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Development of Physical and Optical Methods for In-shell Brazil Nuts Sorting and Aflatoxin Reduction

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Abstract

Physical methods for mechanically in-shell Brazil nut sorting by color, size, density and inner deterioration were developed to assess nut quality and reduce aflatoxin contamination. Nuts were able to be sorted *by color* utilizing the standard shell chromaticity components L*, a* and b* at ranges of 31.51 to 48.64, 4.09 to 10.07 and 11.90 to 22.95, respectively. It produced a homogeneous color batch and segregated the off-standard ones (darkest, lightest and stained). By *size* sorting utilizing three oval sectioned trays with the following dimensions 40x25, 35x23 and 20x19.5 mm [length x width] and vibration, nuts were sorted into three sizes Groups: I, II and III for large, medium and small, respectively. Through *density* sorting the light nuts (shell/nut <1.5) which have inner mass reduction by fungi growth and/or dehydration, were separated with two compressed air streams from the healthy ones. Utilizing near infrared (NIR) spectrophotometry, the *nuts inner deterioration* was detected, with no need of de-shelling them at the wavelength range of 2200 to 2500 nm. Any nut measurement detected, lower or higher than those sorting settings, was considered off-standard and rejected. Pools of the final standard and off-standard nuts were analyzed for aflatoxins by LC-MS/MS. No toxin was detected in the final standard batch up to the method LOQ (0.08, 0.09, 0.10 and 0.12 µg/kg for AFB_1,AFB_2, AFG_1, AFG_2, respectively). On the other hand, the off-standard rejected batch had 16.4 µg/kg of AFB_1. These methods are important tools for building an in-shell Brazil nut sorting machine to assess nut quality and reduce aflatoxin contamination.

Keywords: In-shell Brazil nut, Deterioration, Sorting, Physical methods, Near infrared spectrophotometry, NIR, Aflatoxins

1. Introduction

Prevention of aflatoxin contamination in grains and nuts has not always been effective. The risk of aflatoxin contamination in Brazil nuts can occur, either in the forest or during the storage and it can vary with the year of harvest (Pacheco and Scussel, 2006; 2009). Nut contamination by fungi, especially the aflatoxigenic species of *Aspergillus flavus* is directly related to the climatic conditions (high humidity and heat) in the Amazon forest during the period of harvesting (wet season: December to May) (Pacheco and Scussel, 2006; 2007; 2009; Campos/Pas, 2004). The presence of aflatoxins is a concern for exporters of Brazil nuts especially since 1998, when the European Community (EC) reduced the maximum tolerance limit of total aflatoxins (AFB_1 + AFB_2 + AFG_1 + AFG_2) and AFB_1 to 4 and 2 µg/kg, respectively (EC, 1998; Newing and Harrop; 2000).

Deteriorated in-shell nuts can present characteristics, which can be noted *visually* by consumers, such as visible mould, slimy, low weight, discoloration, irregular forms and, if the nut is rattled or right inside in the shell. Those characteristics can help to select the deteriorated/contaminated nuts from the healthy ones (Marklinder etal., 2005). The visual characteristics of Brazil nuts (size, weight, and colour) observed by consumers may indirectly indicate nut deterioration. However, when utilizing equipment for exact measurements of these nut characteristics, one can establish standards to facilitate the selection of healthy nuts and the rejection of deteriorated and/or contaminated ones. These parameters i.e., the characteristics of Brazil nuts for color, size and weight were measured and standards established (De-Mello and Scussel, 2007).

The knowledge of nuts and grain physical characteristics is essential to project, build and operate cleaning and sorting machines (Teixeira et al., 2003). The aim of the sorting process is to eliminate the grains or nuts with low quality, providing a product with better quality and standardize them in relation to their external characteristics. Furthermore, the sorting process can help to reduce aflatoxin contamination by the rejection of empty and broken grains, and those with color alteration and strange materials (Rohner, 1988; De Oliveira, 2006).

Studies have been reported evaluating the characteristics of deteriorated grains and nuts, in order to obtain data to distinguish them from healthy ones. Corn contaminated with mycotoxins showed lower density than non–contaminated ones (Dowell et al., 2002; Huff, 1980; Pearson and Wicklow, 2006), therefore, they could be separated by flotation in water or sucrose/salt solutions (Huff, 1980; Huff and Hagler, 1982; 1985; Rotter et al., 1995; Shetty and Bhat, 1999) or by air stream to sort the lighter corn from the heaviest ones (Huff, 1980). Pearson et al., in 1994 showed that early split pistachio which is more exposed to fungi and aflatoxin contamination is significantly different in relation to length, width and weight. From these characteristics it was possible to develop an image system for sorting earlier split pistachio nuts (Pearson and Slaughter, 1996).

Dowell et al. (2002), reported that the presence of fungi can affect some grain characteristics such as color, protein structure and oil content. Contaminated grains were rejected by color sorting machines and reduced 51% of the aflatoxin contamination in peanuts followed by hand sorting (Dickens and Whitaker, 1975; Zovico et al., 1999). Color sorting was more effective than fluorescence sorting for decontamination of peanuts (Pelletier and Reizner, 1992), however, fluorescence sorting was effective to reduce levels of aflatoxin contamination in pecans (Tyson and Clark, 1974) and pistachio (McClure and Farsaie, 1980). On the other hand, Steiner et al. (1992) reported pistachios that did not present fluorescence was contaminated with high aflatoxin levels and the opposite for Brazil nuts that presented fluorescence but were not aflatoxin contaminated.

The bright greenish yellow fluorescence (BGYF) was related as an efficient method for grain and nuts decontamination (Shotwell at al., 1975; Pearson et al., 2001), and it has been used by industries for sorting contaminated grains (Palomino et al., 1998).

Nowadays researches have been conducted to evaluate the behavior of near infrared (NIR) spectroscopy in the nuts and grains mycotoxin decontamination. During fungal growth, nutritive substances of the grains and nuts are metabolized and composition modified. Since grains and nuts contain high levels of lipids, the degradation of lipids also can influence the changes of NIR spectra of the nuts (Hirano et al., 1998). In addition, a fungal infected kernel would also scatter more light than a sound vitreous kernel because fungi growth can lead to produce endosperm powdery (Pearson et al., 2001). Wicklow & Pearson (2006) achieved 85% exaction in the selection of corn contaminated with aflatoxin from non-contaminated ones by NIR. Apart from sorting contaminated grains (Hirano et al., 1998), NIR can also be used to detect defects in almonds (Pearson, 1999; Pearson and Young, 2002) and pistachio (Haff and Pearson, 2006) or to predict their internal quality/matrix constituents (Wicklow and Pearson, 2006; Baye et al., 2006; Delwiches, 2003). Other non-destructive methods that have been used to evaluated grains and nuts quality are X-ray, acoustic and imaging (Pearson and Young, 2002; Haff and Pearson, 2007; Cetin et al., 2004; Pearson et al., 2008).

Regarding the Brazil nuts, the current sorting process is carried out manually and it occurs mainly after the first drying step (Pacheco and Scussel, 2009). That step is time consuming, labor intensive and not totally effective as deteriorated nuts are not visible in the in-shell nuts. Therefore, the development of a sorting machine is required.

The aim of this study was to develop physical and optical methods for in-shell Brazil nut mechanically sorting utilizing the nut characteristics (for color, size, density) reported by De-Mello and Scussel (2007), as well as to apply the NIR spectrophotometry for inner nut deterioration (De-Mello and Scussel, 2007) in order to assess nut quality and evaluate their efficiency to reduce aflatoxin contamination for future machine application.

2. Materials and Methods

2.1 Samples

In-shell, processed (dry) Brazil nuts, 65 kg (ca. 7137 nuts) obtained from the city of Manaus, state of Amazonas, Brazil (aflatoxin level: 5.62 µg/kg; moisture content – mc: 6.2 %).

2.2 Chemicals

Methanol and acetonitrile (HPLC grade) from Carlo Erba, ultra-pure water (MilliQ system) from Millipore and amonium acetate (analytical grade) from Vetec.

2.3 Aflatoxin Standards

AFB_1, AFB_2, AFG_1 and AFG_2 (1mg) from Sigma.

2.4 Equipment and Apparatus

Color sorting: Sphere spectrophotometer, with dynamic rotational sampling, model SP60 was from X-Rite. *Size classification:* iron plates (30 x 30 cm); sieves with 500 mm diameter with square sections (38.0x38.0; 25.5x25.5 and 19.0x19.0 mm for large, medium and small nuts, respectively), vertical milling machine, Turret; vibratory engine MR 110 and optic tachometer model TD 713 were from Instrutemp. *Density sorting:* poly(vinyl chloride) (PVC) pipe (75 and 500 mm for diameter and length, respectively); industrial fan was from Werner Hainlin; frequency inverter CFW-04 and anemometer model 445 were from Testo. *Inner nut deterioration analysis:* NIR spectroscope, model 900 PLS (wave length 1100-2500 nm) was from Femto. *Aflatoxin analysis:* by liquid chromatography - tandem mass spectrometry (LC-MS/MS) system comprised of a liquid chromatograph, model 1100 was from Agillent and mass/mass detector API 4000 equipped with electrospray (ESI) and atmospheric pressure chemical (APCI) ionization sources was from Applied Biosystems MDS SCIEX. Column: C_8, particle size 5 μm, 4.6 mm, 150 mm was from Zorbax. *Other materials:* digital caliper (6 inches) was from Lee; semi-analytical scale, model 440-53 was from Kern. Industrial Brazil nut-cracker, provided by CIEX factory, Manaus, AM, Brazil.

2.5 Physical and Optical Methods for In-Shell Brazil Nuts Sorting

The methods for shell color, size and density sorting were developed utilizing the Brazil nut standard characteristics of shell color (L*, a* and b*), nut sizes (length and width of Group I, II and III), weight (shell/nut ratio - S/N) reported by De-Mello and Scussel (2007). The NIR methodology for detecting inner nut deterioration utilized was that reported by the same authors in 2009. The methods are as follows:

Shell Color Sorting. The chromaticity values (achromatic component L* -relative darkness and lightness; chromatic component a* -green to red; chromatic component b*-blue to yellow) were obtained by photo-colorimetric readings on the in-shell nuts utilizing the standard range of 31.51 to 48.64, 4.09 to 10.07 and 11.90 to 22.95, L*, a* and b*, respectively. Shell nut readings higher and lower than those ranges were discarded. Experiments were carried out in triplicates. The batch-rejected nuts were kept for final aflatoxin analysis. The selected nuts were then proceeded for size sorting.

Size Sorting. Using the nut length and width dimensions for Groups I, II and III of 53.2, 43.9 and 36.6 mm and 29.7, 26.5 and 18.8 mm for large, medium and small, respectively (De-Mello and Scussel, 2007), it was possible to study two methods for nut size classification. The first method was carried out utilizing 3 vibratory sieves (diameter 500 mm) with *square sections*: 38x38, 25.5x25.5 and 19x19 mm for the three size groups, respectively. The second method was carried out by building square trays (500 x 500 mm each) with specific sized and shaped sections. The sections were designed in an *oval shape* with the following dimensions for length and width: Tray I (large size) with 40 x 25 mm; Tray II (medium size) with 35 x 23 mm and Tray III (small size) with 20 x 19.5 mm. A fourth Tray was also built with no sections located at the base of the tray system to receive the discarded midget nuts, dust nut debris and other unwanted impurities that could come with the nut batch. The tray system was adapted to a vibratory engine (speed 171.7 rpm) for sorting the size nuts during vibration (Figure 1). Samples (5 groups of 13 kg each ie., ca. 1427 nuts/13 kg) were submitted to size sorting tests with 5 minutes of vibration (n = 5). Only the material that got onto the Tray IV, i.e., that passed through the three previous sectioned trays were kept for aflatoxin analysis. The three size classified nuts were submitted to the next step.

Density Sorting. According to the principle that light (low weight) in-shell nuts can be spoiled, i.e., have inner mass reduction due to fungi proliferation and/or dehydration thus lower weight (shell/nut ratio - S/N<1.5) than the healthy ones (S/N>1.5) reported by De Mello and Scussel (2009), Brazil nuts after being classified by size, were sorted by density difference into (a) light (off-standard nuts = blown off nuts) and (b) heavy (selected nuts = not blown nuts) by applying compressed air. The aim of this sorting process was to reject nuts, which present weight lighter than 5 g (standard nut weight: 12.9, 8.8 and 6.3 g each size, respectively). A prototype system with compressed air with adjusted flow for blowing off deteriorated low weight ones was built using a pipe (diameter 75 mm and length: 500 mm), which was applied to an industrial fan, airflow 5 to 20m/s and engine rotation of 1000 to 3000 rpm (Baye et al., 2006; Delwiches, 2003). The compressed air provided by the industrial fan was canalized through the pipe. A sieve was adapted on the bottom of the pipe in order to hold the heavier nuts. The size classified in-shell Brazil nuts obtained from the previous sorting process were loaded continuously in the pipe and sorted by density. The rejected nuts i.e., the lighter that blew off were separated manually, weighed and kept for aflatoxin analysis. These rejected nuts were also checked by the next sorting process i.e., NIR.

Inner In-Shell Nut Deterioration Detection. The method was carried out by applying the transmittance NIR spectrophotometry analysis in order to detected and segregate the nuts with inner deteriorations (off-standard). Therefore, the three sorting methods selected in-shell nuts were analyzed by scanning individually throughout their inner structures by an emitting NIR frequency. It passed through the whole in-shell nut (crossing one side of the shell through the inner nut endosperm and to the other shell nut side and reflected). It measured the variation in the inner nut structure in comparison to the readings of a standard (not presenting deterioration / healthy) nut (Pearson, 1999; Pearson and Young, 2002; Haff and Pearson, 2006). Thus nuts with wavelength readings between 2200 and 2500 nm that is considered the range for inner-deterioration by De-Mello and Scussel (2009) were segregated. The readings were taken from the three faces of each nut. The selected (no inner deterioration) and rejected (with inner deterioration) batches had individual nuts opened to check deterioration visually, and analyzed for aflatoxin. The rejected nuts from the previous sorting methods (*color, size and density*) were also NIR checked.

2.6 Aflatoxin Analysis

The methodology used was LC-MS/MS-APCI in the positive mode (Xavier and Scussel, 2008). LC conditions: C_8 column, flow rate of 1 ml/min, mobile phase of methanol/water (55:45, v/v) held for 3 min then changed to methanol/water (70:30, v/v) from 3-5 min. MS/MS: the APCI source operated at dissolvation temperature of 700°C. Curtain gas was 15.0 psi, nebulizer gas at 55.0 psi, corona discharge needle current of 5μA and collision-activated dissociation (CAD) 10. The parent (and the two daughters) ions *(m/z*) were selected for each toxin. For aflatoxin B_1, 313.1 *m/z* (241.10; 285.10); aflatoxin B_2, 315 *m/z* (259.09; 287.20); aflatoxin G_1, 329.1 *m/z* (200.05; 243.05) and aflatoxin G_2, 331.2 *m/z* (245.07; 231.20). The LOD and LOQ were obtained by finely grinding, homogenizing and spiking the nuts prior extraction with aflatoxins, at five concentrations ranging from 1 to 10 μg/kg. The method LOD was defined by 3 times the signal/noise ratio and LOQ by 6 times the signal/noise. Five points were used to build an analytical curve, in order to obtain the R values for LOD and LOQ. Each point corresponded to a mean of five injections of each extract. The LOQ obtained were 0.08, 0.09, 0.10 and 0.12 μg/kg for each toxin, respectively and 0.39 μg/kg for total aflatoxins. Aflatoxins were analyzed in the batches (a) prior to the nut sorting; (b) after the total sorting process in a pool of the final: (b.1) selected and (b.2) rejected / off-standard nuts; as well as in the midget remained in the sorting Tray IV. The analysis was carried out in triplicate. The *mc* was by gravimetry (AOAC, 2005). Figure 1 presents the chart flow of the in-shell Brazil nut sorting procedures and the steps that had aflatoxin analysis carried out.

The statistical analysis was carried out by analysis of variance –ANOVA- (Montgomery, 2001).

3. Results and Discussion

3.1 Physical and Optical Methods for In-Shell Brazil Nuts Sorting

The whole parameters of the physical and Optical methods developed for in-shell Brazil nuts sorting by shell color, nut size and density as well as the NIR measurements for nut internal deterioration, are shown in Table 1.

3.1.1 Nut Shell Color, Size and Density

Shell Color Sorting. When the shell color standard ranges for the shell chromaticity components L*, a* and b*, considered as acceptable color, were applied to the Brazil nut, it was possible to select the nuts with the standard, normal shell color (mean: L* 39.46, a* 7.91, b* 17.99). The off-standard ones were discarded i.e., any shell chromaticity component reading that was higher (mean: L* 54.09, a* 20.76, b* 24.93) or lower (mean: L* 4.44, a* 3.08, b* 7.21) than those standard ranges set in the method, were separated from the main batch. The batch had improved its quality showing more homogeneous shell color for better acceptance by consumers. Color apart from nut external quality, may be an indirect way to identify fungal and mycotoxin contamination and a tool to be used for sorting machine development. It also had reduced the probability of having contaminated ones, shell color wise (De-Mello and Scussel, 2007). It was observed that the off-standard nuts presenting the readings for the darker and lighter and so for the stained ones, when the shell was opened they revealed more often deteriorated nuts (1 out of 4 nuts - RSD 39%). On the other hand, deteriorated nuts were found in nuts that had shells presenting the standard color, and healthy nuts were found in stained shells. Therefore, regarding aflatoxin contamination and nut color sorting, the off-color segregation did not present a direct correlation thus was not enough and did not assure safety.

Size Sorting. When nuts were sorted /classified using the sectioned trays, it was possible to separate them into large, medium and small sizes. The nuts had their best size separation through the *oval* shaped *sections* trays than through the *square sectioned* sieves. Brazil nuts have irregular sizes and shapes and it was difficult to sort them through the square sections (38x3; 25.5x25.5 and 20x19.5 mm, respectively for each size). It was also not possible to predict the position and how the nuts would pass through those sections by length (horizontal position) or by width faces (vertical position), leading to blockage. Two or more small nuts getting on the big sections would block them and other nuts could not pass through to the following medium sized sections of Tray II. That also happened in the medium sections; consequently small nuts were wrongly classified as big and medium to the small sizes. Utilizing the *oval* shaped *sections* reduced the size classification error. It was observed that small nuts did not block the larger sections and easily passed through them

without blocking and confusing the size classification. Thus the Brazil nut sizes were more correctly classified (with quite low SD and RDS% ranging from 0.4 to 2.3 and 9.0 to 9.9%, respectively) of the 5 repetitions of the portions (13 kg). The largest nuts (nuts sizes > 50, mean 53.2 mm) didn't pass through the Tray I (40 x 25 mm); the medium nuts (nut sizes: 40 – 50, mean 43.9 mm) passed the first tray but, did not pass through the Tray II (35 x 23 mm), and consequently the smallest nuts (nut sizes: > 20 < 40, mean 36.6 mm) passed the second tray but didn't pass through the Tray III (size: 19.5 x 20 mm). Moreover, through those sections midget nuts (size < 20 mm) and small particles (dust, dirt, pieces of shell) were able to pass through the 3 trays and were received in the non-sectioned Tray IV (Figures 2 and 3). The total time for nut sorting of 30 kg was 5 min. The aflatoxin analysis of the material collected on Tray IV showed that they contained 2.21 μg/kg of AFB_1. In fact those rejected materials can contribute to aflatoxins contamination, as they may contain deteriorated nut debris and aflatoxigenic fungi spores.

Density Sorting. From the range of compressed air speed studied (flow: 5 to 20 m/s) and engine rotations (1000 to 3000 rpm), two standards of air speed for nut density separation was obtained: 10 and 11 m/s, using rotation of 1400 and 1792 rpm, respectively. From these speeds it was possible to sort nuts with mean density 10.34 g (min 5.7, max 15.3) and 8.13 g (min 4.7, max 13.9) with rather low SD and RSD of 3.98 & 8.19% and 4.37 & 9.34%, respectively. For the rejected nuts, they were 9.94 g (min 4.8, max 12.8) and 7.26 g (min 13.2, max 15.8) with SD and %RSD of 4.96 & 3.36% and 6.88 & 4.53% for the two air speeds, respectively. In fact low density (S/N<1.5) nuts may be more prone to aflatoxin contamination than the high density ones (De-Mello and Scussel, 2007) and have been reported by other authors for grains and nuts (Dowell et al., 2002; Huff, 1980; Pearson and Wicklow, 2006). It is important to emphasize that, although, the lightest nuts blew off with the air stream, some heavy (healthy) nuts were also rejected (blown off) during the density sorting. That can be explained for the irregular shape of the Brazil nut. In order to get the Brazil nut floating, the air pushing force needed to be higher than the nut density. However, the pushing force is proportional to the nut geometry (air contact area). In conclusion, some big nuts that had more contact area (positioned during the air flow), could blow off easier and faster than the small ones, even if they were heavier than the big ones. Those rejected big nuts can be, either return to the batch and be density sorted again, or they can go to the factory de-shelling section to be used shelled. Different airflow may be required for each Brazil nut size, in order to avoid the blown off of some heavy (healthy) nuts despite of the sizes. This can be sorted out by utilizing previously size classified nuts.

3.1.2 Inner In-Shell Nut Deterioration

When the three sorting methods selected standard in-shell nuts were scanned individually throughout their inner structures by NIR spectrophotometry, it was possible to identify some nuts with higher wavelength readings at the range 2200 to 2500 nm, corresponding to 5% of the total final sorted batch. That is the range to detect inner nut deterioration (De-Mello and Scussel, 2009). Those nut deteriorations are not visible by human eyes nor can be detectable by the previous sorting methods as the nuts are in-shelled. Those readings are indication that some in-shell nuts were not edible/safe as they were moldy.

That reading variation happens because fungi proliferation affects the nut integrity and its components (lipids and protein) producing a dark powdery endosperm, quite different from the vitreous and homogeneous endosperm nut. Those tissue alterations scatter the NIR beam reducing its transmittance when it crosses the entire in-shell nut material giving readings different of that obtained in the healthy nuts (Dowell et al., 2002; Wicklow and Pearson, 2006; Pearson, 1999; Pearson and Young, 2002).

As far as nut inner deterioration sorting by NIR and aflatoxin contamination are concerned, it was found a rather direct correlation in the nut batch used for this study. When aflatoxins were analyzed in the pool of healthy (no inner deterioration) nuts sorted (by NIR spectrophotometry), no toxin was detected up to the method LOQ in the healthy ones. However, 16.4 μg/kg of aflatoxin B_1 was detected in the off-standard in-shell nuts (with inner deterioration). The final rejected batch concentrated the total aflatoxin content of the initial nut batch. Important to emphasize that the results on nut inner deterioration obtained by nut density (S/N ratio) sorting - De Mello and Scussel, 2007 - with compressed air corroborated to a certain extent to the NIR analysis; however the last (NIR) was more precise. That shows that those sorting methods used previously can still reject healthy nuts. However, they reduce in a certain extent the probability of aflatoxin contamination, even if not precisely. Despite of that the methods highly improved the nut batch visual quality.

3.2 Aflatoxin Levels versus Physical and Optical Methods for In-Shell Brazil Nuts Sorting

No aflatoxin was detected in the final batch of Brazil nut sorted by color, size, density and NIR. They were detected only in the pool of rejected (off-standard) nuts at level of 16.4 μg/kg of aflatoxin B_1 corresponding to ca. 5% of the total initial nut batch (7135 nuts). The *shell color* sorting allowed to reduce the probability of having aflatoxin contamination, as the color off-standard shell nuts when had their shell opened, presented more often deterioration. However, deteriorated nuts were found in shell nuts having standard color, showing that color segregation was not efficient, aflatoxin wise, and did not assure safety. By *size sorting* it was observed that the nuts classified as small (Group III) presented more deterioration and so aflatoxins, however, it was not constant. By *density sorting* the midget nuts were

separated and had aflatoxin contamination thus also allowed to reduce aflatoxins. Finally, by *NIR*, the deteriorated nuts (with possible aflatoxins contamination) were detected more precisely.

The NIR spectrophotometry analysis showed to be the best sorting procedure regarding detection of fungi deterioration and possible aflatoxin contamination with quite high percentage of confidence for the probability of aflatoxin presence. This system is a non-destructive alternative to measure nut deterioration that should be included in a sorting machine (Baye et al., 2006).

Regarding the off-standard nuts that did not meet the standards set through the sorting methods (except for the NIR segregated ones), they could be sent to the Shelling Section of the factory after having their nut quality (deterioration and aflatoxin contamination) inspected by the Quality Control Laboratory. We did not analyse aflatoxins of each nut NIR segregated, separately.

4. Conclusions

The physical methods developed and applied for in-shell Brazil nut sorting were able to assess the quality of the batch studied. They also helped to reduce fungal deterioration and aflatoxin contamination as well as standardized color and size of the nuts. Through rejecting midget nuts, dust, dirt and other small particles, the *size sorting* method lead to some further aflatoxin reduction. Also, by *density* difference due to nut mass reduction by fungi proliferation (<1.5 S/N ratio), deteriorated nuts were able to be segregated. Finally, when *NIR* was used, deteriorated in-shell Brazil nuts were more precisely segregated, and the selected nuts had no aflatoxin detected up to the method LOQ with the advantage of no need to de-shell them.

Brazil nuts can be mechanically sorted by means of those physical methods developed. The nut different characteristics and alterations were able to be distinguished and allowed to separate them into healthy and off-standard nuts. Size, weight, color and NIR methods are going to be gathered in one machine to have nuts sorted automatically for better quality and safety for trade. Other methods such as imaging, X-ray and acoustic will be evaluated in the near future. This is the first work carried out on developing physical procedures for specific Brazil nut sorting.

Despite of the quality improvement of the methods applied to the in-shell Brazil nuts, it would be better nuts to be commercialized shelled, as the spoiled ones could be visually segregated and consumers do not eat then (De-Mello and Scussel, 2007; Pacheco and Scusssel, 2007). It would be better policy.

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Table 1. Physical and Optical Methods Parameter Settings for In-Shell Brazil nut Sorting and Their Application to a Naturally Aflatoxin Contaminated Batch

Physical and optical methods for sorting	In-shell Brazil nut[a, b]									
	Standard[c]					Off-standard[d]				
	Parameter		Range		Nuts	Parameter		Range		Nuts
Shell nut color	*color:*		*chromaticity:*		*(%):*	*color:*		*chromaticity:*		*(%):*
	normal (L*,a*,b*)		L*31.51 - 48.64 a* 4.09 - 10.07 b*11.90 - 22.95		82	lightest/darkest/stained(L*,a*,b*)		L*< 31.51; > 48.64 a* < 4.09; > 10.07 b* < 11.90; > 22.95		18
Nut size	*group:*	*size:*	*oval sections(mm):*	*nut sizes(mm):*	*(%):*	*group:*	*size:*	*oval sections(mm):*	*nut sizes(mm):*	*(%):*
	I	large	40 x 25	53.2 (>50)	58	IV	midget nuts (with deteriorated nut debris, fungi spores and dust)	< 20 x 19.5	<20	4
	II	medium	35 x 23	43.9 (40-50)	31					
	III	small	20 x 19.5	36.6 (>20 <40)	7					
Nut density	*group:*	*density:*	*m/s[e]:*	*nut density(g):*	*(%):*	*group:*	*density:*	*m/s:*	*nut density (g):*	*(%):*
	I+ II + III	heaviest (S/N>1.5)	10[f]	10.34 (min 5.7; max 15.3)	84	I+ II + III	lightest (S/N<1.5)	10	9.94 (min 4.8; max 12.8)	16
			11[g]	8.13 (min 4.7; max 13.9)				11	7.26 (min13.2; max 15.8)	
Inner nut deterioration[h]	*nut condition:*		*λ (nm):*		*(%):*	*nut condition:*		*λ (nm):*		*(%):*
	healthy[i]		1100 to <2200		95	deteriorated[j]		2200 to 2500		5
Aflatoxin[k]			ND[l]					16.4 µg/kg (AFB_1)		

[a] batch 65 kg (7,137 nuts)
[b] total aflatoxins (AFB_1+AFB_2+AFG_1+AFG_2) prior sorting = 5.62 µg/kg
[c] selected nuts
[d] rejected nuts
[e] air stream flow
[f] 1400 rpm engine speed rotation
[g] 1792 rpm engine speed rotation
[h] near infrared (nir) spectroscopy – the standard nuts selected or rejected by the 3 physical methods were submitted to NIR
[i] vitreous endosperm
[j] powdery endosperm
[k] pool of total standard or off-standard nuts analyzed for aflatoxins (after passing through all the sorting processes)
[l] method LOQ: 0.39 µg/kg (LOD: 0.195 µg/kg) for total aflatoxins; method LOQ: 0.08, 0.09, 0.10 & 0.12 µg/kg (LOD: 0.04, 0.045, 0.050 & 0.060 µg/kg) for AFB_1, AFB_2, AFG_1 & AFG_2.

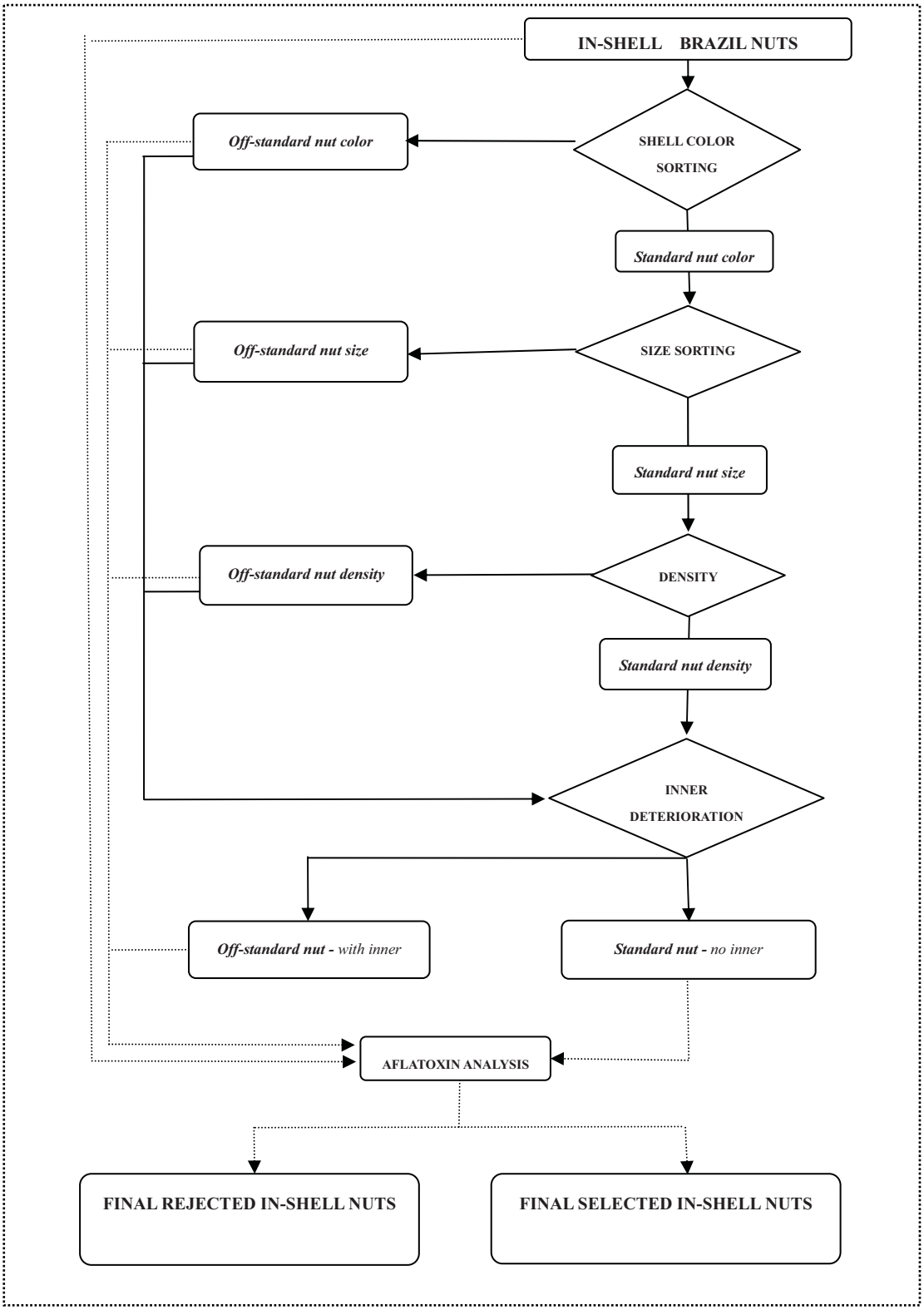


Figure 1. Flow chart of the physical and optical methods applied for in-shell Brazil nuts sorting showing the off-standard/rejected nuts and aflatoxin analysis steps

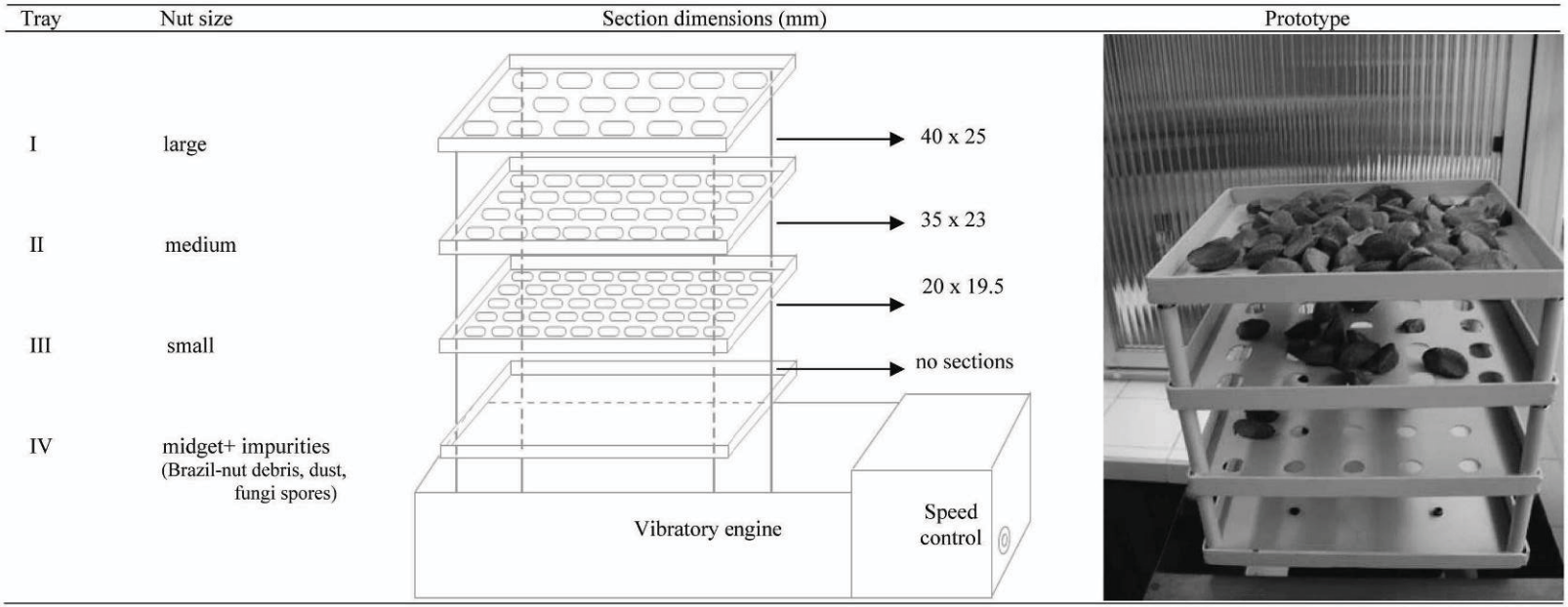
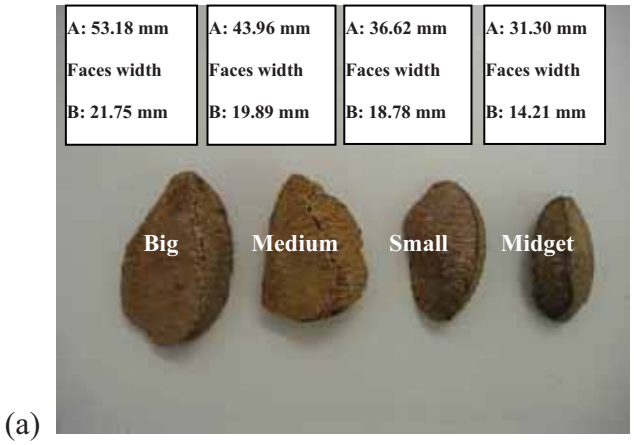
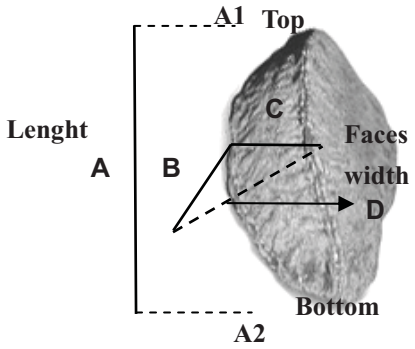


Figure 2. Sectioned vibratory trays for size sorting: (a) scheme of section dimensions for each nut Group (I, II, III) and (b) the prototype used for the in-shell Brazil nuts study



(a)



(b)

Figure 3. In-shell Brazil nuts (a) classified by vibratory trays and (b) points of nut dimensions taken for size tray sections design

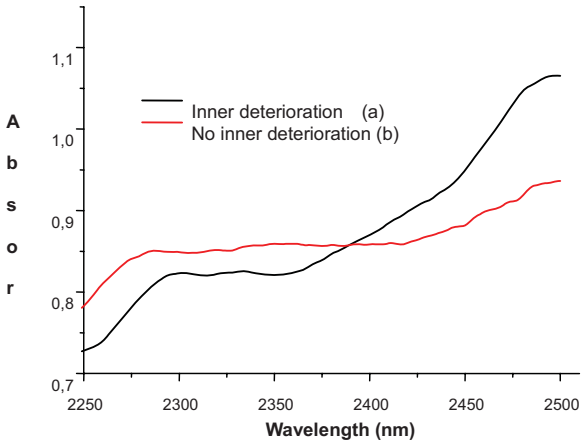


Figure 4. Near infrared curves obtained from two in-shell Brazil nuts showing differences on absorbance intensities: (a) nut presenting inner-deterioration (*off-standard*) and (b) nut without deterioration (*standard*).

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In Vitro Antimicrobial Activity and Fungitoxicity of Syringic Acid, Caffeic Acid and 4-hydroxybenzoic Acid against *Ganoderma Boninense*

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Abstract

This paper discusses the *in vitro* antimicrobial activity and fungitoxicity of syringic acid, caffeic acid and 4-hydroxybenzoic acid which is found in oil palm root. Experiments were observed for fourteen days, repeated at least three times and data were recorded daily. The antimicrobial activities and fungitoxicity of the phenolics against *Ganoderma boninense* were expressed in inhibition of radial growth of *G. boninense* on PDA ameliorated with the three different phenolics with a range concentration of 0.5-2.5 mg/ml. Syringic acid was found to be very fungitoxic to *G. boninense* even at concentration of 0.5 mg/ml, the lowest concentration tested in this experiment. When the concentration is increase to 1.0mg/ml of syringic acid, the pathogen is inhibited. Caffeic acid and 4-hydroxybenzoic acid were having inhibitory effect with the highest concentration tested; 2.5mg/ml strongly inhibited the growth of *G. boninense* in comparison to the control.

Keywords: *Ganoderma boninense*, Syringic acid, Caffeic acid, 4-hydroxybenzoic acid

1. Introduction

Oil palm (*Elaies guineensis* Jacq.) is truly "a golden crop of Malaysia" since it generates profitable export earning for the country and truly nature's gifts for alleviating poverty in Malaysia (Basiron, 2007). The Malaysian palm oil industry is economically big and diversified. Malaysia is currently the world's largest producer and exporter of oil palm. Areas of oil palm have increased from 54,000 hectares in 1960 to 4.05 million hectares in 2005, reflecting a compound annual growth of 10.06%. Production increased from 94,000 tones in 1960 to 15 million tones in 2005, or almost 160 times within 45 years. This represents a compound annual growth of 11.93% per year (Basiron, 2007). The devastating Basal Stem Rot (BSR) disease caused by *Ganoderma boninense* is considered the most serious disease faced by oil palm in Malaysia (Benjamin, 1995). Oil palm has an economic life span of 25-30 years. Basal stem rot can kill more than 80 percent of stands by the time they are half-way through normal economic life (Abdul Razak *et al*., 2004). In the late 1960s and early 1970s in Sumatra, there was little decline in the yield of oil palm until the surviving stand had fallen to about 115 palm/ha, but in more recent plantings, any loss of palm was associated with a loss of yield (Corley and Tinker, 2003). Yield of infected palms was also reduced by 20-40% compared to the year before infection was detected (Khairudin, 1995). Palms with *Ganoderma* yielded between 13 and 21% less than healthy palms at the same age

(Nazeeb et. al., 2000). Heavily infected field yielded 26% less at 11 years after planting, and 46% less at 15 years by which time incidence was 67% (Gurmit, 1991). There is currently no effective cure for *G. boninense* infection in an existing stand. Preventive and ameliorative treatments which are commonly carried out show various degrees of effectiveness (Sariah and Zakaria, 2000). Determination of total phenolic content in *G. boninense* infected and healthy oil palm roots showed susceptible palm roots at week four had low phenolic content, whereas week one had high phenolic content. Gallic acids concentrations decreased in the four weeks old roots of infected susceptible palms compared to healthy roots. Determination of total phenolic content in infected palm seedlings root (DX P) also showed low phenolic content compared to the non infected palm seedlings root. This indicate phenolic compounds are involved in oil palm resistance against *Ganoderma* (Mohamad Arif et. al., 2007).To identify the possibility of oil palm resistance against *G. boninense* in certain circumstances need further investigation. However, if resistance in oil palm against *G. boninense* is possible, it may contribute to tackling the problem. In a collaborative experiment to this research, we have also found syringic acid, caffeic acid and 4-hydroxybenzoic acid present in oil palm roots in natural condition or after elicitation. In this paper, we present the works on *in vitro* effect of syringic acid, caffeic acid and 4-hydroxybenzoic acid to *G. boninense*.

2. Materials and methods

2.1 Ganoderma boninense

Cultures were provided by Borneo Samudera Sdn Bhd, Sabah, Malaysia, maintained at 25^{o}C on Potato Dextrose Agar (PDA).

2.2 In vitro bioassay

A series of 0, 0.5, 1.0, 1.5, 2.0 and 2.5 mg/ml of syringic acid, caffeic acid and 4-hydroxybenzoic acid from Sigma® were ameliorated into the PDA, which the phenolics were first dissolved in Acetone: Water (50:50; v/v). Solvent was served as positive control. The growth of the pathogen was expressed in centimeter of radial growth.

3. Results and discussion

3.1 In Vitro bioassays

In vitro bioassays were conducted to test the fungitoxicity and antimicrobial activities of syringic acid, caffeic acid and 4-hydroxybenzoic acid to *G. boninense*. Syringic acid was found to be very toxic to *G. boninense*. Although at the lowest concentration tested (0.5 mg/ml), *G. boninense* was fully inhibited up to day 5. There was slight non significant increase in the diameter measured of the pathogen at day 6 up to day 15 (Figure 1). Higher concentrations of syringic acid tested (1.0, 1.5, 2.0 and 2.5 mg/ml) totally stopped the growth of the pathogen (Figure 2, 3, 4, 5 & 6). Growths of *G. boninense* both in positive and negative control were not significant to each other indicate the toxicity was coming from the phenolic and not from the solvent. Maximum growths (9cm) were achieved by both positive and negative control within 10 days after incubation. This is in correlation to many works on plants which demonstrated the fungitoxicity effect of syringic acid. In resistance raspberry to fungus *Didymella*, syringic acid was found accumulated in the bordering zone of lesion forming a barrier to the fungus. The *in vitro* fungitoxic of syringic acid was later confirmed to be very toxic at low concentration (Kozlowska and Krzywanski, 1994). In sugar cane, cultivar Mayarí 55–14, which is highly resistant to smut disease showed a major accumulation pattern of syringic acid when interact with the pathogen (de Armas et. al., 2007).

In another hand, pathogen inoculated into agar containing 0.5mg/ml of caffeic acid and 4-hydroxybenzoic acid showed a significantly increased throughout the observation, to a maximum size of the plate at day 11. Surprisingly, in 1.0 mg/ml and 1.5 ml/mg of caffeic acid and 4-hydroxybenzoic acid the growth of this pathogen is greater than control and reached a maximum at day 10 (for 4–hydroxybenzoic acid), but grow slower in 1.0 and 1.5 mg/ml of caffeic acid after day 10 in comparison to control. Both caffeic acid and 4-hydroxybenzoic acid failed to fully inhibit *G. boninense* when the concentration was raised up to 2.0mg/ml. But the pathogen managed only to grow up to 5.58 cm (in 4-hydroxybenzoic acid) and 6.57 cm (in caffeic acid) at last day of observation.

But the effect of 4-hydroxybenzoic acid was obviously inhibitory with the highest concentration tested in this experiment (2.5 mg/ml) which caused the *G. boninense* reached only 3.28 cm in diameter after 14 days. If this concentration is achievable in oil palm root, 4-hydroxybenzoic acid may play a role in the real *G. boninense*-root interaction. The role of this phenolic has been demonstrated in rice hull against various microorganisms. An evaluation of 50% inhibition of growth (IC_{50}) revealed that most of the gram-positive and some gram-negative bacteria were sensitive to 4-hydroxybenzoic acid at IC_{50} concentrations of 100-170 µg/ml (Cho, et. al., 1998). But in caffeic acid the growth of this pathogen continuously to increase significantly after day 6 although in the same concentration as 4-hydroxybenzoic acid, gave a maximum growth of 7.68 cm at day 14. But the growth rate of this pathogen was inhibited by this concentration in comparison to control. This is in accordance to many research that showed caffeic acid is ubiquitously present in plants and a potent phytotoxin affecting plant growth and their physiology (Singh et. al., 2009). In another report, caffeic acid was also found inhibiting the growth of four sweet potato pathogenic fungi and

germination of proso millet seeds in bioassays. Inhibitory activity in the bioassays reported also suggests high periderm caffeic acid levels contribute to the storage root defense chemistry of some sweet potato genotypes (Harisson et. al., 2003).

4. Conclusion

In this paper, we presented the result of *in vitro* experimental investigation on the antimicrobial activity and fungitoxicity of syringic acid, caffeic acid and 4-hydroxybenzoic acid to *G. boninense* inoculated on PDB ameliorated with different concentration of the phenolics respectively. We found syringic acid is the most fungitoxic phenolic among the three, with the lowest tested concentration; 0.5mg/ml can inhibit the growth of *G. boninense* while 1.0 mg/ml totally killed the pathogen. On another hand, the highest tested concentration of caffeic acid and 4-hydroxybenzoic acid; 2.5 mg/ml only inhibited the growth of *G. boninense* in comparison to the control.

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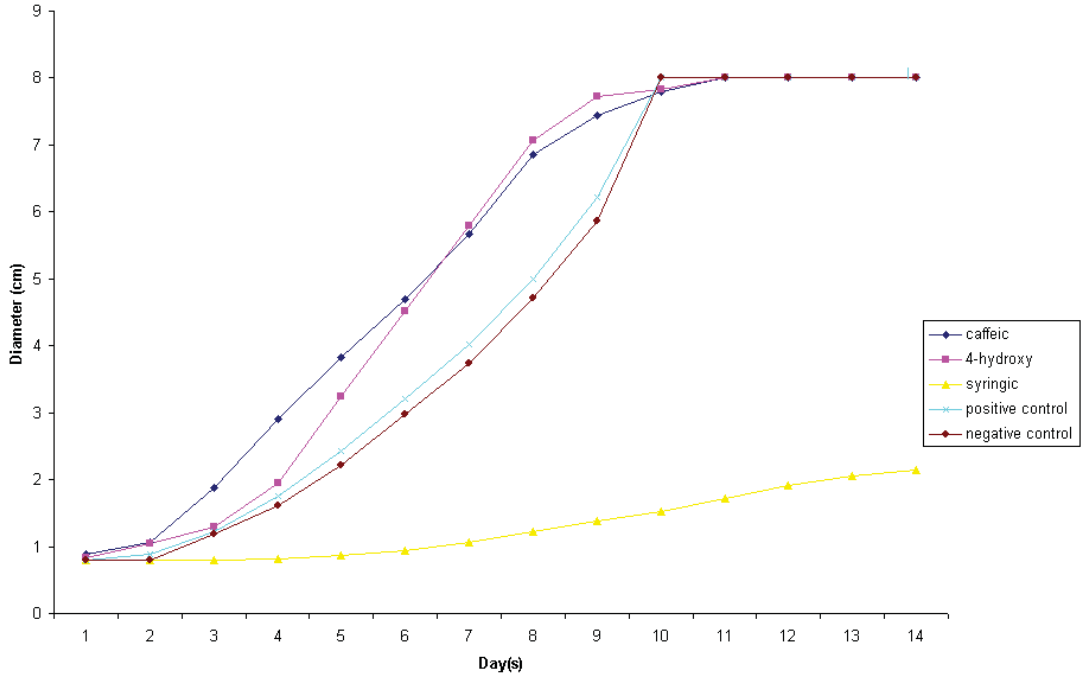

Figure 1. Radial growth of *Ganoderma boninense* on PDA ameliorated with 0.5 mg/ml of three different phenolics.

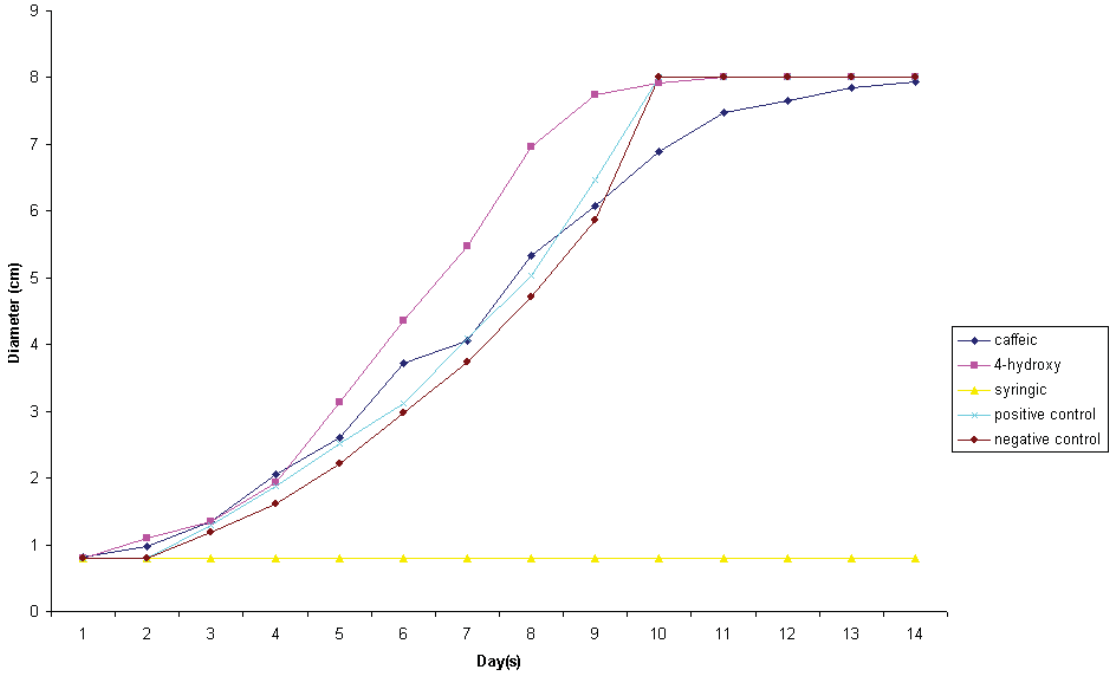

Figure 2. Radial growth of *Ganoderma boninense* on PDA ameliorated with 1.0 mg/ml of three different phenolics.

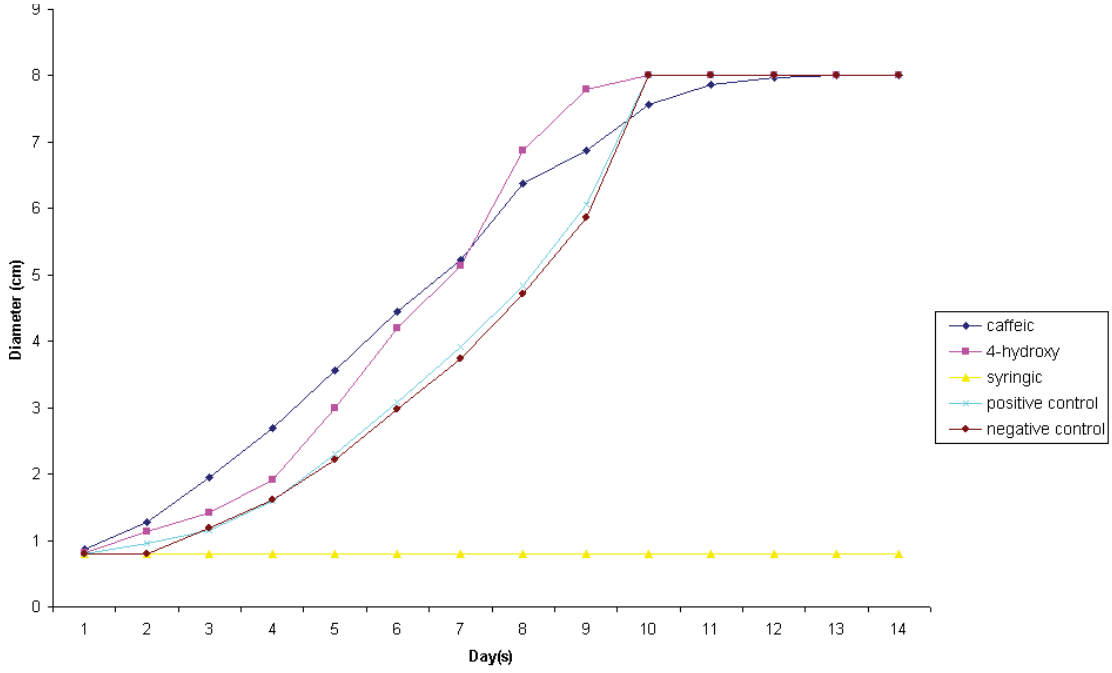

Figure 3. Radial growth of *Ganoderma boninense* on PDA ameliorated with 1.5mg/ml of three different phenolics.

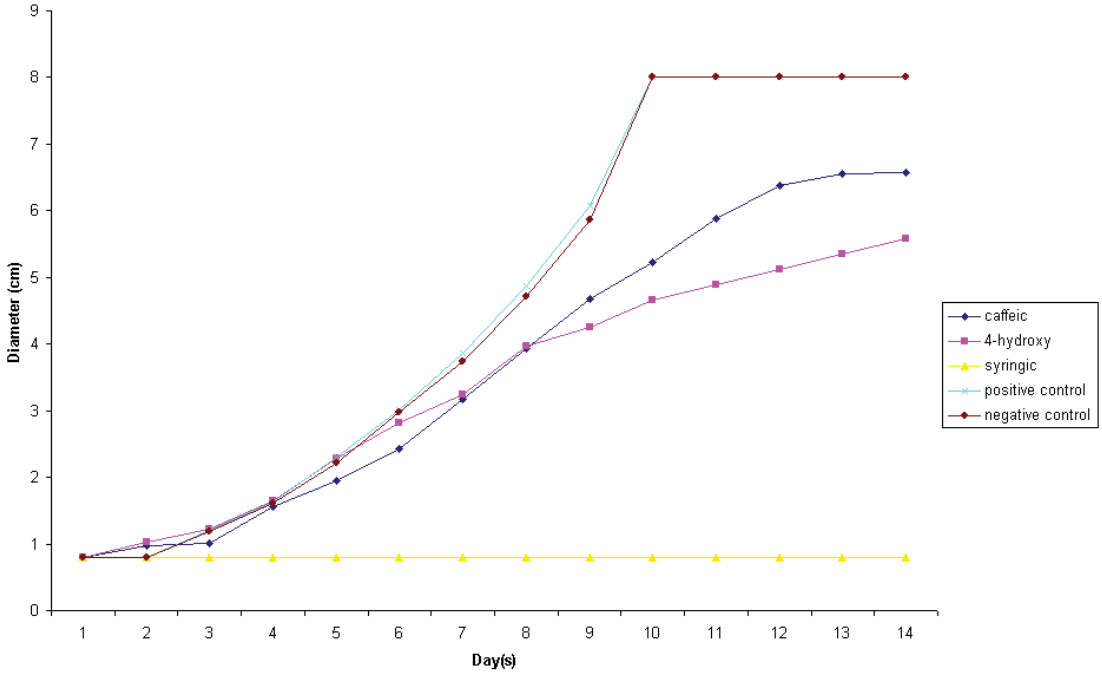

Figure 4. Radial growth of *Ganoderma boninense* on PDA ameliorated with 2.0 mg/ml of three different phenolics.

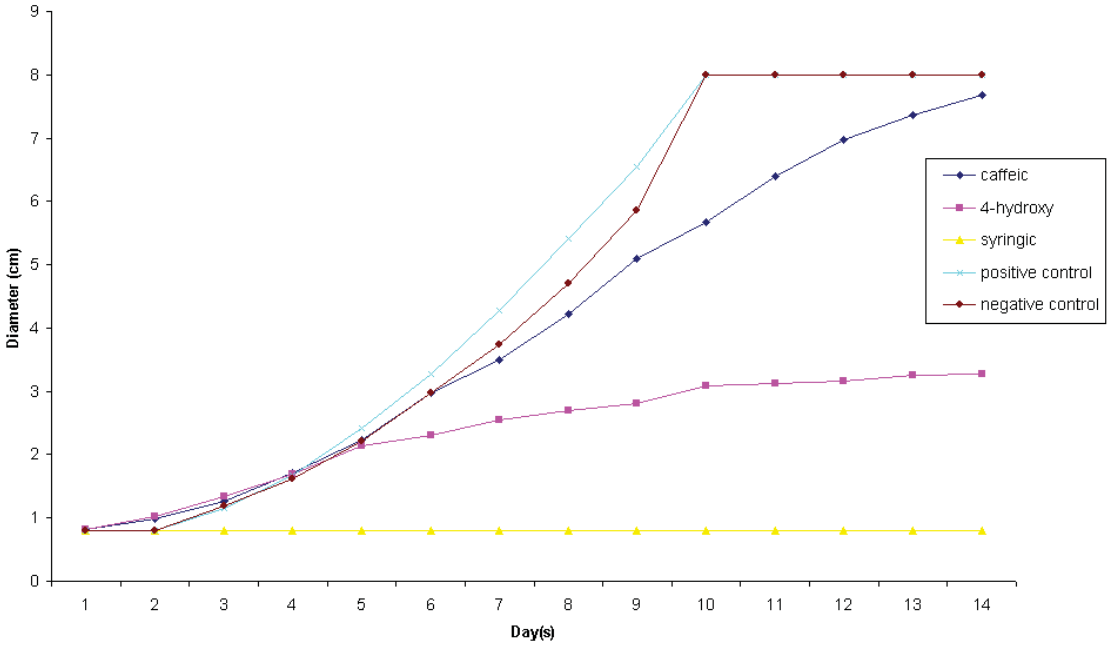

Figure 5. Radial growth of *Ganoderma boninense* on PDA ameliorated with 2.5 mg/ml of three different phenolics.

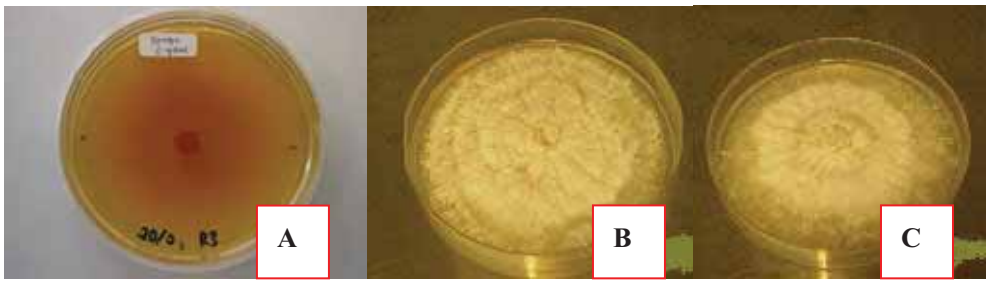

Figure 6. *Ganoderma boninense* failed to grow after incubated for 14 days in 1.0 mg/ml of syringic acid ameliorated in PDA and killed in this concentration (A). Continuous growth both in positive control (B) and negative control (C)

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Application of *Enterococcus Faecalis* FK-23 in Fattening of the Japanese Black Cattle

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Abstract

Objective: By investigating the effects of feeding *Enterococcus faecalis* FK-23 preparation to Japanese Black cattle in the late fattening period, this paper discusses the increased value of beef and the feasibility. Method: 10 beef cattle were grouped into the Test Group and the Control Group, 5 beef cattle in the Test Group were fed *E. faecalis* FK-23 preparation at 8.0 g per day per head. The experiment included weight measure, blood test for White Blood Cell (WBC), Vitamin A (VA), Vitamin E (VE), Total Cholesterol (TC), glutamic oxaloacetic transaminase (GOT), blood urea nitrogen (BUN) and blood glucose (GLU), and HPLC test for fatty acid composition in neutral fat. Results: Comparing to the Control Group, the Test Group had excessive appetite, appreciable increase of daily gain (DG) ($p<0.05$), appreciable increase of serum VE and TC ($p<0.05$), about the same level of VA, and obvious tendency of increased fatty acid in neutral fat, and showed an end weight increase of 4.3% and carcass weight increase of 9.3 kg. Conclusion: Feeding *E. faecalis* FK-23 preparation to Japanese Black cattle in the late fattening period adds additional value to the beef cattle and improves its productivity.

Keywords: *Enterococcus faecalis* FK-23, Anti-oxidation, Meat quality, Fattening, Vitamin A, Vitamin E

1. Introduction

In Japan, the quality grade of beef determines the price of carcass; especially BMS (Beef Marbling Standard) number contributes 80% of the decision-making factors (Hirooka, H., 1998, p. 22-28). In order to increase BMS number, VA is usually controlled in fattening. There are abundant reports on the correlation between BMS and serum VA concentration. Recently, it is believed that the meat with the best quality, i.e. so called marbled beef with the ideal BMS number, can be obtained when serum VA concentration of cattle is kept at lower level from month 15th to month 24th (Nade, T., 2007, p. 161-166; Okumura, T., 2006, p. 387-393; Nakanishi, N., 2002, p. 273-282; Oka, A., 1998, p. 90-99). In addition, influencing factors, such as Vitamin E, Vitamin C, Se, cholesterol and genetic gene, are also widely reported (Nade, T., 2008, p. 495-466; Watanabe, D., 1999, p. 119-128; Noguchi, T., 1978, p. 125-130; Mitsumoto, M., 1992, p. 47-53; Watanabe, D., 1999, p. 195-202; Ichijo, S., 1993, p. 109-114; Buckley, D. J., 1989, p. 1193-1197; Itoh, M., 2003, p.

43-49; Oka, A., 1999, p. 137-144; Mori, M., 2006, p. 15-19). New functions of VE found by Evans et al, the so-called fertility vitamin, have recently received continuous attentions. The meat quality of cattle with low concentration of serum VA, but continuously low concentration of TC and VE, is not good (Watanabe, D., 1999, p. 119-128). VE, existing in the membrane lipid of organism, can prevent membrane lipid from peroxidation (Noguchi, T., 1978, p. 125-130). It also has strong anti-oxidation and may maintain freshness of meat (Mitsumoto, M., 1992, p. 47-53), and can protect liver (Watanabe, D., 1999, p. 195-202), prevent muscle from denaturation (Ichijo, S., 1993, p. 109-114), etc. It is believed that oxidation of meat begins with lipid-rich membrane (Buckley, D. J., 1989, p. 1193-1197). By combining with radical after entering into cell membrane, the VE directly inhibits oxidation of pigment and lipid, as well as indirectly keeps reduction activity of Metmyoglobin to maintain the color of meat and the stability of lipid (Mitsumoto, M., 1992, p. 47-53). TC concentration in the late fattening period exhibits a significant positive correlation with BMS number, carcass weight (CWT), rib-eye area and rib thickness (RT), but a significant negative correlation with BCS (Beef Color Standard) number (Itoh, M., 2003, p. 43-49). Although lowering serum VA concentration can increase BMS, cattle thereupon develops the symptoms of VA deficiency, such as anorexia, blindness, ataxia and etc., in turn uptakes less feed and feed efficiency decreases (Oka, A., 1999, p. 137-144). However, researches on the color of meat directly related to the benefits of manufacturer and consumer are still lagging behind.

Lactic acid bacteria *E. faecalis* FK-23 strain comes from intestine of healthy individuals. The heat-processed product (FK-23 preparation) has tumor resistance (Ohasi, K., 1993, p. 396-399), infection resistance (Nohmi, T., 1996, p. 323-328), as well immunity activation (Ohashi, K., 1992, p. 919-925; Hasegawa, T., 1996, p. 103-112; Kanasugi, H., 1996, p. 563-565), etc. It is safe and non-toxic (Shimada, T., 1998, p. 53-60).

By feeding FK-23 preparation to Japanese Black cattle in the late fattening period, we investigated the effects of FK-23 preparation on the fattening and meat quality grade, assessed the added value of beef, and studied the practical applications of FK-23 preparation in the fattening of Japanese Black cattle.

2. Materials and methods

2.1 Materials

This experiment was carried out at the fattening farm of Japanese Black cattle in Mie Iga City from October 2006 to April 2007. 10 female Japanese Black cattle in the late fattening period, same habitat, same paternal line, 22 months old, were grouped into the Test Group (5 cattle) and the Control Group (the other 5 cattle). Fattening feed and fattening procedure were in accordance with the established procedure of the farm. FK-23 preparation is a dried dead bacteria powder of heat-processed pure *E. faecalis* FK-23 strain, provided by Nichinichi Pharmaceutical Co., Ltd. (Japan).

2.2 Methodology

Begin with body weight determination and venous blood collection of both groups, feed the Test Group with FK-23 preparation mixed into feed at 8.0 g per day per head; then collect venous blood once every month and weigh once every three months. At the time of slaughtering after six months of experiment, test carcass weight, collect subcutaneous adipose and renal adipose, and determine the quality grade of meat. Based on the blood morphology, count the white blood cell with a fully-automatic MEK-6258 White Blood Cell Counter manufactured by Nihon Kohden Corporation of Japan and test. the white blood cell percentage with an Olympus AH-3 optical microscope. Based on the blood biochemical analysis, test VA and VE with Shimadzu LC-6A HPLC made in Japan under the conditions that column is Shim-pack CLC-ODS (6×150 mm), liquid phase is methanol, flow rate is 1.5mL/min, and detection wavelength is 326 nm. Determine total cholesterol (TC), glutamic oxaloacetic transaminase (GOT), blood urea nitrogen (BUN) and glucose (GLU) by colorimetric assay on Fuji Dry Chem 3000V from Japan respectively with TCHO-P III Kit Fuji Dry Chem slide from Japan, GOT/AST-P III Kit Fuji Dry Chem slide from Japan, BUN-PIII Kit Fuji Dry Chem slide from Japan and GLU-P III Kit Fuji Dry Chem slide from Japan. Test the composite and the weight percentage of fatty acid in subcutaneous adipose and renal adipose by GC method with Shimadzu GC-2010, wherein the saturated fatty acid includes myristic acid, palmitic acid and stearic acid, and the unsaturated fatty acid includes myristoleic acid, palmitoleic acid, oleic acid, linoleic acid and linolenic acid.

Compare and study the differences of carcass assessment results between the Test Group and the Control Group based on the appraisal report of meat quality grade from Japanese Meat Grading Association.

Based on the statistical analysis, take analysis of variance on the changes of body weight, daily gain, VA concentration and VE concentration with StatView Ver.5.0 from SAS Institute of Japan; carry out the two group paired-samples t-test and compute the Pearson's Correlation Coefficient of blood biochemical experimental data.

3. Results

3.1 Body weight and daily gain (DG)

There are no significant statistical differences ($p>0.05$) in body weight change and daily weight gain between two groups. However, the test group, after having taken FK-23 preparation for three months, has the trend that its daily gain

is greater than the control group's (p1=0.20 and p2=0.19) (Table 1).

3.2 Experiments based on the blood morphology and blood biochemical analysis

The test result based on the blood morphology does not show any abnormality. The results of t-test and Pearson's Correlation Coefficient based on the blood biochemical analysis indicate that there are significant statistical differences ($p<0.05$) in VE concentration (Figure 1) and no statistical differences ($p>0.05$) in VA, GOT, BUN, GLU and WBC (Table 2) between two groups. Table 3 exhibits the Correlation Coefficient (r) between two groups resulted from the correlation analysis of blood biochemical data. From table 3, we can see a low positive correlation (r=0.250) between VA and VE of the Control Group and a low negative correlation (r=-0.214) between VA and VE of the Test Group. Although there are no statistical differences ($p>0.05$) in TC concentration between the Test Group and the Control Group, TC concentration of the Test Group shows a gradual increasing tendency. In the Control Group, VA shows a low negative correlation with GOT (r=-0.352) and BUN (r=-0.241); VE shows a positive correlation with TC (r=0.532), a low positive correlation with WBC (r=0.212), no significant correlation with GOT, BUN and GLU ($r<|0.20|$); TC shows a low positive correlation with GOT (r=0.362) and GLU (r=0.291); GOT shows a low positive correlation with GLU (r=0.331) and WBC (r=0.267); BUN shows a low negative correlation with GLU (r=-0.362), a positive correlation with WBC (r=0.325). In the Test Group, VE shows a positive correlation with TC (r=0.763), no significant correlation with GOT, BUN, GLU and WBC ($r<|0.20|$); TC shows a low positive correlation with BUN (r=0.360), a low negative correlation with GLU (r=-0.331); GOT shows a low negative correlation with BUN (r=-0.212) and GLU (r=-0.410), a positive correlation with WBC (r=0.687); BUN shows a negative correlation with GLU (r=-0.528) and WBC (r=-0.321).

3.3 Analysis on the composite of fatty acid

There are no statistical differences ($p>0.05$) in saturated fatty acid and unsaturated fatty acid of neutral fat between two groups. But the proportion of unsaturated fatty acid in both subcutaneous adipose and renal adipose of the Test Group shows increasing tendency (Table 4).

3.4 Carcass assessment score

There are no statistical differences ($p>0.05$) in carcass weight and meat quality grade between two groups. But the carcass weight, rib-eye area, yield estimated percentage and rib thickness of the Test Group is higher than these of the Control Group (Table 5).

4. Conclusion and discussion

Although controlling intake of serum VA can increase BMS, cattle thereupon may develop typical symptoms of VA deficiency, such as night vision blindness, weight loss, limbs edema, etc. It in turn has a strong negative impact on fattening effectiveness and markedly decreases the value of carcass (Yano, H., 2004, p. 79-104). Influence of VA to the weight gain includes not only food intake, but also feed efficiency (Oka, A., 1999, p. 137-144). Feeding with food lack of VA for a long time may cause the decrease of VA concentration in blood and liver, and decrease of albumin and cholesterol. Moreover, it may have a negative effect on the quality of beef (Hodate, K., 1999, p. 22-28).

Physiological activity of VE can be summarized in the following aspects: (1) Anti-oxidation. Capturing the active oxygen resulted from oxidation of highly unsaturated fatty acid in lipid assures the normal functions of cell. (2) Anti-aging. Located near to the phospholipid membrane in biofilm structure of cell, VE can keep the safety of biofilm by preventing lipid from oxidation and resist aging. (3) Enhancement of Immunity. VE can enhance the defense response of body, the response of humoral immunity (increase of IgG) and cellular immunity (increase of mitogen) to the lymphocyte stimulation, the phagocytosis and bactericidal effect of eosinophils, and the immunity by reducing the cortisol of body. (4) Regulation of endocrine. Activate pituitary gland and adrenal gland to secrete hormones. (5) Improvement of blood circulation. Activate microcirculation to prevent ischemia (Ichijo, S., 1993, p. 109-114). Food intake has a major effect on serum VE and TC, in turn affects the quality of beef. Therefore, it is necessary to enhance the control of nutrition of cattle to improve the meat quality (Watanabe, D., 1999, p. 119-128).

Mayanagi, A. et al (1994, p. 39-47) reported that the fattening method of lowering VA concentration may easily cause VA deficiency and decrease the concentration of VE in blood, observed mainly in the appearance of liver function damage and pneumonia. After slaughtering, the amount of abandoned livers due to abscess, necrosis, etc. showed a negative correlation with VE concentration in blood. Therefore, it is necessary to keep optimal serum VA and VE concentration in blood to improve reproduction and disease resistance of cattle, and establish an economic and effective fattening system.

Mitsumoto, M. et al (1991, p. 1489-1492; 1995, p. 2289-2294) reported that VE, with its strong anti-oxidation, could prevent raw beef from fading and lipid from oxidation during deepfreeze or exhibition. Intake of FK-23 preparation could stabilize the color and lipid of raw beef. This method is safe, practical, and has huge industrialization significance.

Watanabe, D. (1999, p. 119-128) proposed that serum TC concentration of ranch herd with high A5-occurrence rate was

higher than 130 mg/dl. Yano, H. et al (2004, p. 79-104) suggested that serum TC concentration showed a positive correlation with BMS. Serum TC concentration of cattle with higher meat quality grade had increased continuously within 20 months or longer. Therefore, serum TC concentration is considered as an index for evaluating the meat quality. For the Test Group in this study, serum TC concentration has gradually increased to 193 mg/dl after having FK-23 preparation for two months. It indicates that intake of bacteria preparation with high amount of lipid has an indirect positive effect on BMS number, carcass weight, rib-eye area, rib thickness and BCS number.

This study was carried out under the strict control of VA concentration. The horizontal translocation of VA concentration (Figure 1) indicates that intake of FK-23 preparation does not have any effect on VA concentration. There are no statistical difference ($p>0.05$) in body weight and daily gain between two groups. However, after three months of experiment, the Test Group shows a tendency that its daily gain is higher than that of the Control Group. Meanwhile, the average end weight of the Test Group is 4.3% higher than that of the Control Group; and its average carcass weight is 9.3 kg higher than that of the Control Group (Table 1). It is believed that FK-23 preparation can relieve or ameliorate the symptoms such as anorexia and decrease of feed intake due to VA deficiency during the fattening process of Japanese Black cattle. Comparing to the Control Group, VE concentration of the Test Group shows a markedly increasing tendency, and has statistical significance ($p<0.05$). Other relevant correlation analysis results demonstrate that the Correlation Coefficients (r) of VE with VA and TC of the Control Group are 0.205 and 0.532 ($p<0.05$) respectively; while these of the Test Group are -0.214 and 0.763 ($p<0.05$) respectively. VE of the Control Group shows a low positive correlation with VA; however becomes a low negative correlation with VA after having FK-23 preparation. This indicates that VE concentration decreases with the decrease of VA concentration during common fattening procedure; however, after having FK-23 preparation, VE concentration still keeps an increasing tendency even if VA concentration decreases. VE concentration significantly correlates with TC concentration in both groups, there are statistical differences ($p<0.05$) both in VE and TC between two groups, and their correlation in the Test Group is higher than that in the Control Group. In addition, BUN has a Correlation Coefficient of -0.362 and -0.528 ($p<0.05$) with GLU respectively in the Control Group and the Test Group; GOT has a Correlation Coefficient of 0.267 and 0.687 ($p<0.05$) with WBC respectively in the Control Group and the Test Group. All above correlation analysis results prove that feeding FK-23 preparation to Japanese Black cattle in the late fattening period can not only improve serum VE and VC concentration, but also enhance the relation among the correlated indexes. But its mechanism is still unknown.

The analysis on the composite of fatty acid demonstrates that there are no statistical differences ($p>0.05$) in the composite of fatty acid in both subcutaneous adipose and renal adipose between the two groups. But the proportion of unsaturated fatty acid of the Test Group shows an increasing tendency (Table 4). In subcutaneous adipose, the proportion of oleic acid, a component of soft adipose with low melting point (13.3 ℃), increases by 0.8%. Oleic acid has the functions of anti-arteriosclerosis, anti-oxidation, anti-hypertension, anti-aging, anti-senile dementia, anti-constipation, resisting coronary heart disease, inhibiting low density lipoprotein, etc; the proportion of Myristoleic acid, another component of soft adipose, increases by 1.0%. On the other hand, the proportion of palmitic acid, a component of hard adipose with high melting point (82.9 ℃), decreases 0.6%. The total rate of change is 1.2%. The total rate of change of components of fatty acid in renal adipose is 2.6%. The melting point of adipose shows a high correlation with the composite of fatty acid, a negative correlation with unsaturated fatty acid, a positive correlation with saturated fatty acid (Kobayashi, M., 2006, p. 521-527). The proportion of unsaturated fatty acid has no relations with the amount of crude fat, body weight, height and BMS number, but is seriously affected by the breeding bull. Partial substitution of the fine fodder has some effects on the composite of fatty acid in renal adipose, but has no effect on daily weight gain and carcass characteristics (Shinoda, M., 2007, p. 201-208). BMS and carcass characteristics show independent genetic correlations with the melting point and composite of fatty acid (Inoue, K., 2008, p. 1-8). This study was carried out on the same patrilineal Japanese Black cattle under the same conditions of fattening in the same fattening time. The increase of the proportion of unsaturated fatty acid is believed to be correlative with intake of FK-23 preparation, but does not have any effect on the carcass characteristics.

There are no statistical differences ($p>0.05$) in quality grade of carcass, BMS and other evaluation results of meat quality between two groups. But the carcass weight, rib-eye area, yield estimated percentage and rib thickness of the Test Group is slightly higher than these of the Control Group. The proportion of unsaturated and saturated fatty acid has minor change, but its effects can not be identified by naked eyes of the meat appraisers. It has not been reported so far as to the change range of proportion that affects meat evaluation grade and mouthfeel. Therefore, it is important to study the chemical components that affect the flavors and flavor developments of marbled beef.

Feeding FK-23 preparation to Japanese Black cattle in the late fattening period can increase serum VE and VC concentration, improve appetite and feed intake, as well as increase daily gain, end weight and carcass weight. It has some minor effects on adipose characteristics in body, but the proportion of unsaturated fatty acid shows an increasing tendency. Therefore, FK-23 preparation not only increase the economic benefit of fattening farm, but also has no negative effect on the traditional fattening system that depends on controlling VA concentration in feed to obtain marbled beef with the most ideal BMS number. VE-rich meat has a longer shelf time and color retention because of

anti-oxidation of VE. Such a meat with above additional values should be favored by consumers and market. In this study, VE-rich beef is obtained by feeding FK-23 preparation. It is proved that FK-23 preparation can increase the beef's additional value and improve its productivity in the late fattening period of Japanese Black cattle. The effects on the meat tenderness and flavor from the point of view chemical composites should be studied in the future.

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Table 1. Body weight change and daily gain of sample cattle (means ± standard deviation)

Time interval for measurement	*Changes of body weight (kg)*		*DG (kg)*	
	Control Group	Test Group	Control Group	Test Group
At the time of introduction	281±12	281±8	ND	ND
At the beginning of experiment	551±33	542±39	0.69±0.06	0.67±0.08
After three months of experiment	589±31	598±28	0.42±0.18*	0.62±0.12*
At marketing time	636±40	663±28	0.53±0.22**	0.72±0.14**

* represents p1; ** represents p2; ND represents no test on it.

Table 2. Test results based on blood biochemical analysis (means ± standard deviation)

	Item	*Month zero*	*Month 2nd*	*Month 4th*	*Month 6th*
Control Group	VA	65.3±6.9	47.8±9.1	42.2±7.1	48.4±6.4
	VE	298.4±35.4	255.7±16.9	256.3±73.8	319.3±85.6
	TC	147.6±35.1	142.4±14.8	134.0±21.2	168.0±25.1
	GOT	60.0±6.0	69.0±6.8	62.0±7.5	70.0±15.4
	BUN	18.6±2.3	17.3±1.3	20.1±3.4	18.7±1.8
	GLU	67.0±2.8	73.0±6.8	62.0±4.0	68.0±5.9
	WBC	9820±1110	9220±1260	8300±1470	8720±1820
Test Group	VA	60.4±14.2	44.0±5.4	43.8±7.3	51.4±4.9
	VE	229.3±53.3	203.5±73.8	339.1±50.7	386.1±44.0
	TC	130.0±25.1	124.0±32.1	166.0±15.2	193.0±13.4
	GOT	70.0±15.3	71.0±17.3	71.0±14.8	73.0±15.5
	BUN	17.1±2.5	17.0±2.0	21.1±4.9	18.8±3.5
	GLU	65.0±4.8	72.0±4.5	58.0±4.2	66.0±2.6
	WBC	8700±2390	8160±1750	7820±1530	7920±1970

n=10

Table 3. Correlations among test results based on blood biochemical analysis

	F-VA	*F-VE*	*F-TC*	*F-GOT*	*F-BUN*	*F-GLU*	*C-VA*	*C-VE*	*C-TC*	*C-GOT*	*C-BUN*	*C-GLU*
F-VA	-	-0.214	0.101	-0.214	0.054	0.098						
F-VE	-0.214	-	0.763*	0.042	0.186	-0.088						
F-TC	0.101	0.763*	-	0.153	0.360	-0.331						
F-GOT	-0.214	0.042	0.153	-	-0.212	-0.410						
F-BUN	0.054	0.186	0.360	-0.212	-	-0.528*						
F-GLU	0.098	-0.088	-0.331	-0.410	-0.528*	-						
F-WBC	-0.326	0.007	0.012	0.687*	-0.321	-0.189						
C-VA							-	0.250	0.018	-0.352	-0.241	-0.163
C-VE							0.250	-	0.532*	0.029	0.045	-0.006
C-TC							0.018	0.532*	-	0.362	-0.072	0.291
C-GOT							-0.352	0.029	0.362	-	-0.025	0.331
C-BUN							-0.241	0.045	-0.072	-0.025	-	-0.362
C-GLU							-0.163	-0.006	0.291	0.331	-0.362	-
C-WBC							0.070	0.212	0.005	0.267	0.325	0.134

* represents $p<0.05$; C- represents the Control Group; F- represents the Test Group; WBC represents number of white blood cell; Correlation is represented by the Correlation Coefficient (r) of various index between the Test Group and the Control Group.

Table 4. Analysis on the composite of fatty acid in body adipose (means ± standard deviation)

		Saturated fatty acid (%)			Unsaturated fatty acid (%)				
Types of fatty acid		Myristic acid	Palmitic acid	Stearic acid	Linolenic acid	Myristoleic acid	Palmitoleic acid	Oleic acid	Linoleic acid
Subcutaneous adipose	Control Group	2.1±0.2	23.9±1.4	5.2±0.9	0.0±0.0	2.5±0.3	9.8±1.3	54.2±2.5	2.4±0.7
		31.3±2.3			68.9±2.3				
	Test Group	2.0±0.1	23.3±0.7	4.5±0.7	0.0±0.0	2.6±0.3	9.8±1.0	55.0±1.8	2.7±0.4
		30.1±1.5			70.1±1.6				
Renal adipose	Control Group	1.9±0.2	24.4±1.7	22.9±3.4	ND	0.4±0.1	1.7±0.3	46.6±4.3	2.0±0.5
		49.4±5.1			50.7±5.1				
	Test Group	1.8±0.4	23.0±2.3	21.8±3.1	ND	0.4±0.1	1.8±0.2	48.9±5.3	2.2±0.4
		46.8±5.4			53.3±5.4				

n=10; ND represents no test on it.

Table 5. Assessment on carcass (means ± standard deviation)

	Control Group	*Test Group*
Carcass weight (kg)	393.0±30.0	402.3±32.2
Quality Grade	3.2±0.8	3.6±0.5
Rib-eye area (cm^2)	57.3±8.7	61.3±11.3
RT (cm)	6.9±0.5	7.0±0.3
SFT (cm)	2.2±0.6	1.8±0.7
Yield estimated percentage (%)	74.6±1.3	75.4±1.8
BMS number	4.0±1.4	4.8±1.8
BCS number	4.4±0.5	4.8±0.8
BFS number	3.0±0.0	3.2±0.4

n=10; RT represents rib thickness; SFT represents subcutaneous fat thickness; BMS represents beef marbling score; BCS represents beef meat color score; BFS represents beef fat color score.

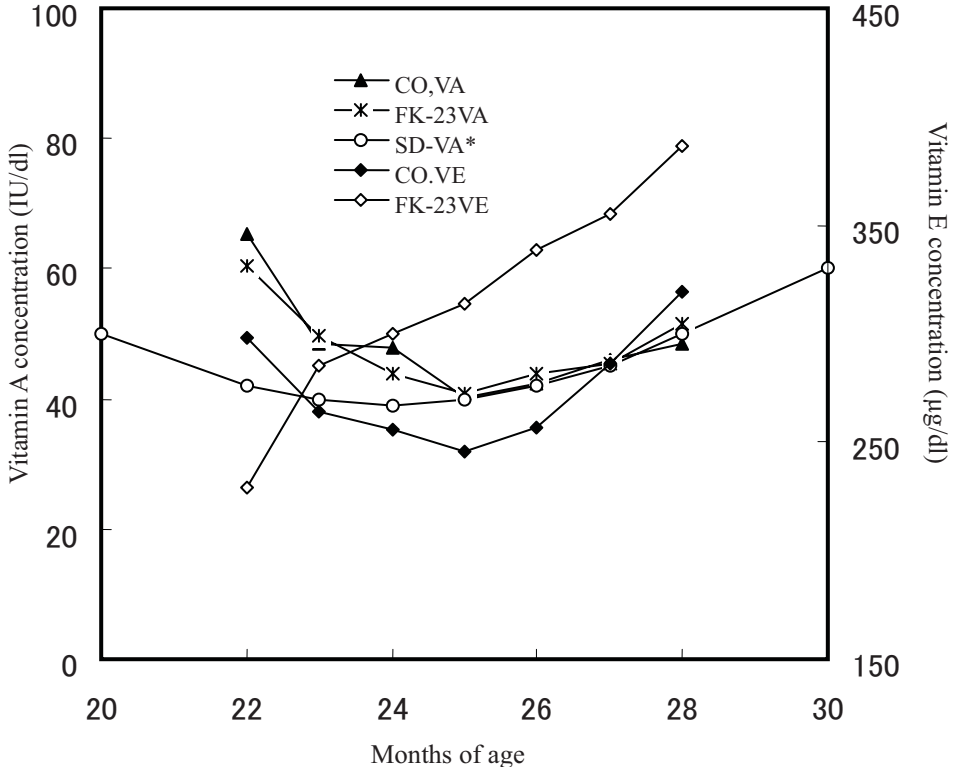

Figure 1. The effects of *E. faecalis* FK-23 preparation on serum VA concentration and VE concentration

Horizontal translocation result indicates that serum VA of the Test Group is similar to that of the Control Group, although decreases relatively late, finally reaches the standard level of concentration. After one month of experiment, VE has continuously increased until marketing time, and there is a significant statistical difference ($p<0.05$) in VE concentration between the Test Group and the Control Group.

FK-23 VA represents VA concentration of the Test Group; CO, VA represents VA concentration of the Control Group; SD-VA* represents the standard VA concentration in cattle blood popularized in fattening Japanese Black cattle (Oka, A., 1999, p. 16-18); FK-23VE represents VE concentration of the Test Group; CO, VE represents VE concentration of the Control Group.

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Antimicrobial Properties of Stem Bark Extracts of *Ximenia Americana*

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Abstract

Ximenia americana is endemic in Northern Nigeria, and has been reported to be used in treatment of various types of ailments. In the present study, the antimicrobial properties of the stem bark extracts of *Ximenia americana* were screened against *Escherichia coli, P.aeruginosa*, *Staphylococcus aureus, P. vulgaris, Candida albicans, B. subtilis* using the disc diffusion method. The result revealed the methanolic and water extract showed significant ($P<0.05$) broad spectrum activity on the growth of many of the test organisms (*E. coli,* and *P. vulgaris, S.aureus, P. aeruginosa* and *B. subtilis*), while the butanolic extract had little activity. Phytochemical screening revealed the presence of alkaloids, saponins, flavonoids, cardiac glycosides, terpenoids and tannins. The study supports the traditional usage of this plant by herbalist as remedy in curing microbial infections.

Keyword: Ximenia americana, *Antmicrobial activity*, Microoganisms

1. Introduction

Traditional medicine is well recognized in Africa and many rural communities rely on the use of ecofriendly and easily available plants in the treatment of a wide variety of ailments. *Ximenia americana* (Olacaceae) which is also known as "wild olive" in English, is a shrub like plant found in abundance in the West African region. It flowers usually in the second part of the dry season, producing cream – white to greenish yellow flowers. The fruits are green but turn golden-yellow or red. The fruit when eaten is very refreshing and has almond acid taste. In Northern Nigeria, the plant is found in the Savannah areas. It is extensively used among the Hausa/Fulani as herbal remedies in treatment of malaria, leproutic ulcers and skin infections of mixed origin (Ogunleye and Ibitoye, 2003). The leaves have been reported to have antibacterial activity (Ogunleye and Ibitoye, 2003) while leaves have been reported to be used in the treatment of fever, tuberculosis, stiffness, Onchocerciasis tooth decay and wounds (Abonnier, 2004). Abonnier, (2004) have reported the roots to be used in treatment of leprosy, syphilis, dysentery, and wounds. The stem bark has been reported to have antitrypanosmal activity (Maikai *et. al*., 2008). The stem bark and the leaves also are used in treating headaches and mumps. The present study is aimed at investigating the antimicrobial activity of the stem bark extracts of *Ximenia americana* .

2. Materials and methods

2.1 Collection of plant Material

The stem bark of *Ximenia americana* was collected from Afaka village 35km to Kaduna (11^o 10' N, 7^o 38' E) in the month of August 2006 and taken to Department of Biological Sciences herbarium, Ahmadu Bello University Zaria for

identification the voucher No. 1612 was deposited. The stem bark was dried at room temperature before crushing into powder using an electric blender and then stored in air tight container and kept at 4^0c until needed.

2.2 Solvent extraction

The powdered material (200g) was shaken in 300ml of acetone: water (2:1) and further extracted according to Latte (1999) to obtain fractions of different polarities. The extracts included petroleum ether extract (PEE), chloroform extract (CFE), butanol extract (BE), methanol extract (ME) and water extract (WE). Each extract was evaporated to dryness using a hot air oven set at 35^0C. The dried extracts were weighed and then stored in air tight container and kept at 4^0c until needed.

2.3 Phytochemical screening of extracts

Phytochemical analyses were done using standard procedures (Harborne 1973; Trease and Evans, 1989; Sofowora ,1993).

2.4 Organisms

The species of bacterial organisms were *Staphylococcus aureus, Pseudomonas aeruginosa, E. coli, bacillus subtilis,Proteus vulgaris* and *Candida albicans*. They were clinical isolates obtained from Ahmadu Bello University Teaching Hospital, Shika Zaria. The cultures of bacteria were maintained on nutrient agar slants at 4^0C, re –identified by biochemical tests (Cheesburgh, 1982) and sub cultured unto nutrient broth for 24h prior to testing.

2.5 Antimicrobial screening

The PEE, BE, ME, and WE extracts (100mg) were dissolved in 1ml of (DMSO, Merck, Germany) and diluted to a final concentration of 20mg/ml; other concentrations were made (15, 10 and 5mg/ml) by serial dilutions. The solutions were sterilized by filtration through a 0.45µm membrane filter. Sterile 9mm discs were impregnated with 50µl of extract and placed on the surface of agar plates inoculated with a microbial culture. Each extract was tested in triplicate. Ciprofloxacin (40µg/disc) served as positive control for the bacteria, whereas amphotericin B (25µg/disc) was control for *C. albicans*. The plates were incubated at 37^0C for 24hours. Antimicrobial activity was recorded if the zone of inhibition was greater than 9mm.

2.6 Statistics

One way analysis of variance (ANOVA) was used to test for level of significance.

3. Results

The results of phytochemical screening of stem bark solvent extracts of *X. americana* are presented in Table 1. The three solvent extracts revealed the presence of flavonoids, saponins, tannins and terpenoids. However, alkaloids, pylobatanins and cardiac glycosides were absent in the water extracts. The antimicrobial screening of the extracts showed that the activity of methanolic and water extracts appeared to be more significant ($P < 0.05$) broad spectrum effect against the growth of *E.coli, P. auruginosa, P. vulgaris, S. aureus* and *B.subtilis* than the butanolic extract at 10, 15 and 20mg/ml concentrations. The results indicate that the organisms were susceptible at higher concentration of the extract (20mg/ml). The extract also showed some activity against *C.albicans* at 20mg/ml while ciprofloxacin the standard drug for bacteria, failed to inhibit the growth of *C. albicans*. In general the bacteria were found to be more sensitive to the extract.

4. Discussion

Microbial infection involving microorganism's poses a very serious public health problem all over the world especially in resource poor African countries. To provide scientific support on the use of *X. americana* traditionally in the treatment of infections we tested the different solvent extracts on some common pathogenic organisms. Extracts of *X. americana* which was tested for antibacterial activity was found to have broad spectrum effect on the growth of many of the test organisms, *E. coli,* and *P. vulgaris, S.aureus, P. aeruginosa* and *B. subtilis*) . The results tend to agree with similar work (Msonthi, 1986; Vlietinck *et al*., 1995; Akiniyi *et al.*, 1996; Ogunleye and Ibitoye, 2003; Rabe and Van Staden, 1997; Buwa and Stada, 2006; Mohanasundari *et al.*, 2007).

The reasons for the differential sensitivity pattern between gram-positive and gram- negative bacterial strains could be due to the outer phospholipids membrane with the structural lipopolysaccaride components, which make their cell wall impenetrable to antimicrobial agents (Nikaido and Va'ara, 1985), while the gram positive bacteria is more susceptible having only an outer peptidogycan, which is not an effective permeability barrier (Scherer and Gerhardt, 1971). Ximenia americana exerted more broad spectrum inhibitory activity against the microorganisms. Our results showed that there was some little activity of the extract on *C. albicans*, a fungus at a higher concentration. This also tends to agree with similar work (Balakrishna *et. al*., 2000) who reported that alcoholic extracts of *Solanum trilobatum* were effective on fungi at higher concentrations. Though the extract concentration was high (20mg/ml) when compared to the concentration of the antibiotic (Ciproflaxicin) which was 40µg. This could be explained that the extracts were still in

their crude form made of complex composition of chemicals compared to the standard drug which was a pure compound. Further purification of the extracts could lead to isolation of purer compounds with increased microbial activity. The medicinal value of plants lies in some chemical substances produced by these plants, these chemicals called "secondary metabolites" include; alkaloids, flavonoids, tannins and phenolic compounds (Cowan, 1999). The mechanisms of action of the chemical constituents of the extracts are difficult to speculate at this point, however, it is known that many antibacterial agents exhibit their actions either by inhibiting the synthesis of the cell wall, nucleic acid, or blocking the synthesis of proteins. Flavonoids have been reported (Dixon *et al.*, 1983; Hostettman *et al*., 1995; Tsuchiya *et. al*., 1996) to complex with extracellular and soluble proteins and to complex with bacterial cell walls. Tannins have the ability to inactivate microbial adhesions,enzymes, cell envelope transport proteins and also complex with polysaccharide (Ya *et. al*., 1988; Scalbert, 1991). Terpenoids have also been (Tassou *et al*., 1995; Taylor *et al*., 1996) reported to be active against bacteria, the mechanism of action involve membrane disruption by the lipophilic compounds. The results of the present study support the use of *X. americana* as having antibacterial properties that can be used as agent in new drugs for the therapy of infectious diseases caused by pathogens. The phytochemicals can further be isolated and undergo further pharmacological evaluation.

4.1 Conclusion

This study justifies the claimed uses of the *X. americana* in the traditional system of managing various infections diseases caused by microorganisms. The herbalists simply use water as the medium of extracting the active principles.

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Table 1. Phytochemical screening of stem bark extracts of *X. americana*

Extract	Alkaloids	Anthraquinones	Cardiac glycosides	Flavonoids	Pylobatannins	Saponnins	Tannins	Terpenoids
Butanol extract (BE)	+	-	+	++	+	++	++	++
Methanolic extract (ME)	++	+	++	++	++	+++	+++	+++
water extract (WE)	-	+	-	+++	-	++	++	++

+++ - highly present, ++ - moderately present, + - faintly present, - absent

Table 2. Antimicrobial activity of stem bark extracts of *X. americana*

Extract/drug	Extract conc. (mg/ml)	Zone of inhibition (mm)					
		Escherichia coli	Pseudomonas aeruginosa	Proteus vulgaris	Staphylococcus aureus	Candia albicans	Bacillus subilis
Butanol extract (BE)	5	-	-	-	-	-	-
	10	-	-	-	-	-	-
	15	-	10	5	8	-	10
	20	7	12	7	11	-	14
Methanol extract (ME)	5	-	-	-	-	-	-
	10	6	10	11	9	-	8
	15	10	15	15	13	-	9
	20	18	17	18	15	8	14
Water extract (WE)	5	-	-	-	-	-	-
	10	9	7	10	8	-	10
	15	14	12	14	14	-	13
	20	19	17	19	20	12	19
Ciprofloxacin	40µg/disc	37	40	38	38	0	40
	25 µg/disc	0	0	0	0	28	0
Amphotericin							

- no activity

Values greater than 9mm indicate activity

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Soil and Agricultural Capability of UiTM Sarawak Campus Farm, Malaysia

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Abstract

An area of 40.5 ha at UiTM Sarawak, Samarahan Campus farm was surveyed at a detailed level. There are three soil major groups, three soil families and six soil series identified in UiTM Sarawak, Samarahan campus. The three soil major groups are Red Yellow Podzolic (RYP), Gley and Organic. The three soil families are BEKENU and MERIT Family of RYP Group, BIJAT and TATAU Family of Gley Group and ANDERSON Family of Organic group. The soil series identified are Bekenu of BEKENU Family, Merit and Jakar of MERIT Family, Daro of BIJAT Family, Tatau of TATAU Family and Gadong of ANDERSON Family. The majority of the area is classified as having agriculture capability of Class 2t, 3te and 4te. Class 2t, 3te, 4te consist of Jakar, Merit and Bekenu series and which are restricted for agriculture due to the steepness of the slope. Class 3wi consists of Daro series soil where wetness and inundation hazard is a moderate limitation for agriculture use. Class 4fw consists of Tatau series soil which is not suitable for agriculture used due to serious fertility and serious wetness. Class 04go consists of Gadong series which is also not suitable for agricultural use due to high groundwater table and undecomposed organic surface layer.

Keywords: Soils, Agriculture, Soil capability, Soil classification, Campus farm, UiTM Sarawak

1. Introduction

The Soil Classification System of Sarawak was updated in 1982 (Tie, 1982). The nomenculture of this Classification System was strongly influenced by the earlier soil classification system (Soil Survey Staff, 1966, Andriesse, 1972; Scott, 1973; and Lim, 1975); Soil Taxonomy (USDA, 1975} and by new finding on soils of Sarawak (Andriesse, 1975; Eilers and Loi, 1978; Loi, 1980; Lah 1980 and Teng 1981).

UiTM Sarawak Samarahan campus is divided into three major physiographic types i.e. hilly areas, undulating areas and low lying areas such as alluvium and waterlogged peat swamp. About 60% of the survey area, UiTM farm, is undulating with a slope ranging from 2-20 degrees. The remaining 40% is flat and low lying, consisting of alluvium plain and basin swamp. Soils occurring in the area are classified based on the current soil classification developed by Teng (2004) and Tie (1982). According to the Sarawak Soil Classification System (Teng, 2004), the diagnostic horizons/characteristics are used for classifying the soils of the Sarawak at the Group level. The diagnostic horizons are adopted as defined in soil taxonomy, 1988. The diagnostic horizons are generally subsurface horizons underlying an epipedon or a leaf litter or at the surface of a partially truncated soil. They are mostly B horizons but may also include part of the A horizons.

The purpose of classifying land according to its capability for agriculture is to provide one of a series of inventories for rational land use planning. The land capability class indicates the severity or degree of limitation in land use while the subclass indicates the specific kind of limitation encountered. Complex technical information contained in soil survey maps and reports, as well as crop yields and fertilizer response data gleaned from research station and statistical reports are presented in simple terms of how suitable the land is for agriculture. Since few figures are available on the crop yield potential of the various soils in Sarawak, the main sources of reliable information are the soil surveys. By means of descriptions, classification and mapping, these provide the basic information on which soils can be rated and grouped into broad capability classes from good to poor. The kind of terrain and the observable soil profile characteristics such as soil depth, color, structure, texture, wetness and parent material are examined and evaluated in agronomic terms of rooting volume, tilth, moisture-holding capacity, drainage, fertility, slope and suitability for mechanization that enhance or limit their potential use. In a land capability map for agriculture, these factors will determine the kinds of limitations to be encountered while the severity of limitation will determine the class rating. Matching the class rating and the kinds of limitations expressed as subclass with the stated requirements of each crop can show the user the types of agricultural crops suitable for each area, the problems likely to be encountered and feasibility of making improvements.

The main objective of this study is therefore to identify the various soil types of the UiTM Sarawak, Samarahan campus farm and to develop recommendations and guidelines for soil management, as reference for land utilization for crop suitability and agricultural potential in the farm.

2. Methods and materials

2.1 Soil survey

The project was a detailed soil survey which comprises preliminary studies of the area, actual field work, laboratory analysis, mapping and assessment of land capability for agricultural crops. In the preliminary studies, previous semi detailed reports and maps of the Samarahan area provided useful information for compiling tentative soil maps on a scale of 1:25,000.A detailed soil survey was carried out at a scale of 1:4,000. The cut lines (rentises) were planned in grid system as shown in Fig 1 so as to cut across as many as soil boundaries as possible.

A grid system of transect lines (rentises) spaced at 100 m interval was used. The field work involved cutting traverses along fixed compass bearings. Along each rentis the soils were examined at 125m intervals to a depth of 125cm using an Edelman auger for mineral soil and for peat soil the soils were examined at a depth of 200cm by using a peat auger. The soil samples were taken along each rentis at every 125 m interval. The slope, vegetation and other land features were also recorded along the rentis. Geostatistical Positioning System Trimble (GPS) was used to locate and identify the soil pit profiles and also for the land use mapping of the survey area. The information from the GPS was transfer to the Geographic Information System (GIS) for mapping.

2.2 Soil sampling

There were 51 soil samples from auger profiles and 3 water samples collected for the determination of chemical and physical soil properties such as pH, moisture content, CEC, etc. General description of the soil such as color, mottle, texture, consistency, stone, depth and others were also taken during the soil sampling,. The soil samples were collected at the sampling point by using an auger at a depth of 0-25 cm, 25-50 cm and 50-75 cm. The soil samples were collected every 125m along the rentis plan, while some soil samples were taken randomly due to no rentises have been cut in the area.. Groundwater samples were taken randomly for lowland soil classification based on the electrical conductivity measurement. A total of seven soil profiles or pit were dug in the farm for a detailed soil description. The soil profiles were described from the surface until a depth of 100 cm except for the organic soil which were described until 150 cm depth.

2.3 Soil analyses

Soil samples were analyzed by the Chemistry Laboratory, Agriculture Research Center Sarawak and also by a private accredited science laboratory. The analyses involved mineral content, particle size distribution, soil pH, moisture content, total organic carbon, total nitrogen, exchangeable cation, cation exchange capacity, reserved element, total P, available P, reserve P and total S. Soil pH was analysed in 1:1 soil to water by using pH meter; moisture content and

mineral content was automated using Thermogravimetric Analyzer; total organic carbon and total nitrogen were analysed by Dumas method and total organic carbon was measured by Multiphase Carbon Determinator LECO RC-412; exchangeable cation and cation exchange capacity reserved element such as Ca and Mg extraction were by hydrochloric acid method and measured by Inductive Coupled Plasma (ICP-AES); total P was determined by perchloric acid and digestion method; available P was determined by Bray II extracting solution, both using UV spectrophotometer; reserve P by using HCl extractable and measured by ICP-AES; and total S by Dumas method and measured by CNS analyzer.

3. Results and discussion

3.1 Soils Series Classification

Soils occurring in the survey area are classified based on the current soil classification (Teng, 2004).The three soil major groups are Red Yellow Podzolic (RYP), Gley and Organic. The three soil families are BEKENU and MERIT Family of RYP Group, BIJAT and TATAU Family of Gley Group and ANDERSON Family of Organic group. The three soil major groups are Red Yellow Podzolic (RYP), Gley and Organic. The three soil families are BEKENU and MERIT Family of RYP Group, BIJAT and TATAU Family of Gley Group and ANDERSON Family of Organic group.

There are three (3) soils groups found in the area and are classified at family and series level as shown in Figure 2 below. Soils of the Merit series are derived from shale parent material. They are mature soils that show increase in clay content with depth and moderately well drained to well drained soils. The Merit soils have a yellowish brown (10YR or yellower), with an A horizon of approximately 20 cm with clay loam texture, weak fine crumbly structure and friable to firm consistence. This horizon is well rooted with few large roots, and fauna activity is high. The B horizon is colored brownish yellow, with clay texture, firm consistence and structure is moderately coarse sub angular blocky. Movement of material from the upper horizon is shown by the presence of organic material along root channels. In the lower part of this horizon, the soil becomes massive and faint mottles occur and there are many soft to hard iron concretions in the B horizon. The BC horizon comprises a mixture of weathered rock (shale) fragments. Meanwhile, soils of the Jakar series are similar to that of the Merit series but differ only in color. The Jakar soils are mainly colored strong brown or redder soil color, with hues of 7.5YR throughout the one meter control section. The Jakar soils have a yellowish brown A horizon but at few sites, the A1 horizon has a darker color of dark brown or dark yellowish brown. The B horizon is more variable. The predominant color is reddish yellow. This reddish yellow or strong brown color having a hue of 7.5YR must occur within 50 cm which is distinctive characteristic of this Jakar series, separating it from the Merit. The texture of the Jakar soils is invariably clay loam A horizon overlying clay B horizon. The soil consistence is friable in topsoil and firm in the subsoil. The weak fine angular blocky structure in the surface horizon is followed by moderate coarse sub angular blocky structure and many iron concretions and grit in the underlying horizons. The soils have low permeability and are normally well drained. Mottles are not common in the Jakar series. They are present only at a few sites and are often associated with the weathering shale fragments.

On the other hand, soils of Bekenu series are well drained soils and have formed in fine grained sandstones. The Bekenu soils profile has a yellowish brown fine sandy loam A horizon overlying brownish yellow fine sandy clay loam B horizon. In the lower part of the subsoil the texture is clay or sandy clay in places. The brownish yellow colour of the B horizon often grades to reddish yellow at depth. The consistence is friable in the upper half portion of the profile but firm in the lower portion. The structure is weak fine angular blocky in the topsoil and weak medium angular or subangular blocky in the subsoil. Weathered fine-grained sandstone and occasionally siltstones fragments are common in the subsoil. The amount of rock fragments is controlled mainly by the underlying rocks and topography. The Bekenu series in the UiTM farm occurred in association with Merit and Jakar series of MERIT Family on undulating to moderately dissected terrain. The Daro series soil in this farm is characterized by clayey texture (.35% clay) throughout the soil profile (100cm), presence of sulphidic materials at a depth of more than 75cm, non-saline groundwater salinity which is less than 1000 micromhos/cm, at 25 degrees C and poorly drained. The Daro series in this area has been reclaimed, where the sulphidic materials have oxidized to form a jarosite upon continued drainage. On the other hand, the Tatau series soil in this farm is characterized by a loamy sand texture. The soil has a light matrix colour of 10YR 6/2 or light brownish grey to 10YR 7/2 or light grey throughout the profile within 100cm. The Tatau series is differentiated from other series in the TATAU Family by the salinity of the groundwater. The soil is non saline soil with groundwater salinity is less than 1,000 micromhos/cm 25^{o}C and poorly drained. The Gadong series in this area consists of more than 150 - 200 cm of residual organic materials overlying a clayey mineral substratum, very poor drainage and a water table generally occurs near or above water surface. A Gadong series soil is an autochthonous organic soil material with a high ash content which is in between 10-35%.

3.2 Agriculture Capability Classification

In the agriculture capability classification, which rates land according to their limitations for agricultural development, there are five classes each for mineral soils and organic soils. The limitations of land characteristics to agricultural use are divided into five levels of severity ranging from none to very serious. The rating of capability classes are based on the number and intensity of the limitations imposed by various land characteristics such as topography, wetness,

physical soil conditions and soil fertility as shown in Agriculture Capability Classification (Figure 4) of the UiTM Sarawak Samarahan Campus farm.

Class 2t, 3te and 4te consists of Merit, Jakar and Bekenu series soils which occur on a wide variety of terrain. Steepness of slopes (c) and erosion hazard (e) are the major limitations to agriculture use. These soils are rated as Class 2t with slopes of 6-12 degrees, class 3te with slopes of 12-25 degrees and class 4te with slopes of 25-33 degrees. Meanwhile, the Class 3wi consists of Daro series soil where wetness (w) and inundation hazard (i) is a moderate limitation of the use of the land for agriculture. In addition, class 3wi is affected by other possible moderate limitations including dense massive clay subsoil (c) and depth of organic surface (o). Although few crops area adapted to poorly drained conditions of the Daro series, it is one of the best natural soils for wetland rice in Sarawak. On the other hand, Class 4fw consists of Tatau series soil where serious fertility (f) and wetness problem (w) which restrict the use of the land for agriculture. In addition, class 4fw is affected by other possible moderate limitations including moisture holding capacity (m), organic surface (o) and inundation hazard (i). The soil classified in this class has such limitations that they are only suitable for a few crops, the yield is low or the risk crop failure is high. However, Class 04go land consists of Gadong series which is very poorly drained peat deeper than 100cm. The most serious limitations include poor drainage due to high groundwater table (g) and undecomposed fibric surface layer. Deep peat is a serious limitation to rooting because it offers only a poor anchorage medium for plant roots. Peat subsides rapidly upon drainage which further increases the difficulty of drainage and the risk of flooding. Gadong series soils are suitable only for wetland rice and sago in their natural states. Major improvements in flood protection, drainage and water table control could change the rating of these soils from 04 to 03. With comprehensive fertilizer and production program a wide range of crops can be grown.

4. Conclusion

The distribution of the soil types in the farm is based on the terrain of the area. The RYP soil group occurred on undulating hill comprising of Merit and Jakar series of MERIT Family and Bekenu series of BEKENU family. The Gley soil group was developed on lowland area or at floodplain areas and are comprised of Daro and Tatau series of BIJAT Family, while Organic soil group can be found in basin swamps which are comprised of Gadong series of ANDERSON Family.

Agricultural capability of the area is predominantly classified in Class 2t-3te-4te and Class 3wi. Class 2t-3te-4te consists of Jakar, Merit and Bekenu series and it is restricted for agriculture due to the steepness of the slope. Jakar, Merit and Bekenu soils that are classified in Class 2t and 3te are suitable for agricultural activities and can be planted with various types of crops such as upland rice, banana, sugarcane, cocoa, oil palm, pepper, papaya, coffee, fruit trees, coconut, cashew and rubber. The 4te class of Jakar, Merit and Bekenu needs proper soil management due to the long term effects of potential soil erosion. It is suitable for only an infrequent crop of hill rice or for small-holder rubber with a permanent ground cover.

Class 3wi consists of Daro series soil and naturally is suitable for wet padi cultivation. However the area now is planted with oil palm, coconut, coffee and guava, because the area has been under continuous drainage. Class 4fw consists of Tatau series soil which is not suitable for agriculture use due to serious fertility and serious wetness, but with proper water and fertilization management the soil can be planted for coconut and cashew. Class 04go consists of Gadong series which is also not suitable for agriculture use but with good water management the area can be improved to Class 03o which can be planted with sago.

Soil analytical data of this area have been analysed for soil classification, but further investigation is needed on the soil fertility of the area. Experimental plots could be built for such a study. It is suggested and recommended that further research should be done on soil fertility or soil quality study in this area in the near future.

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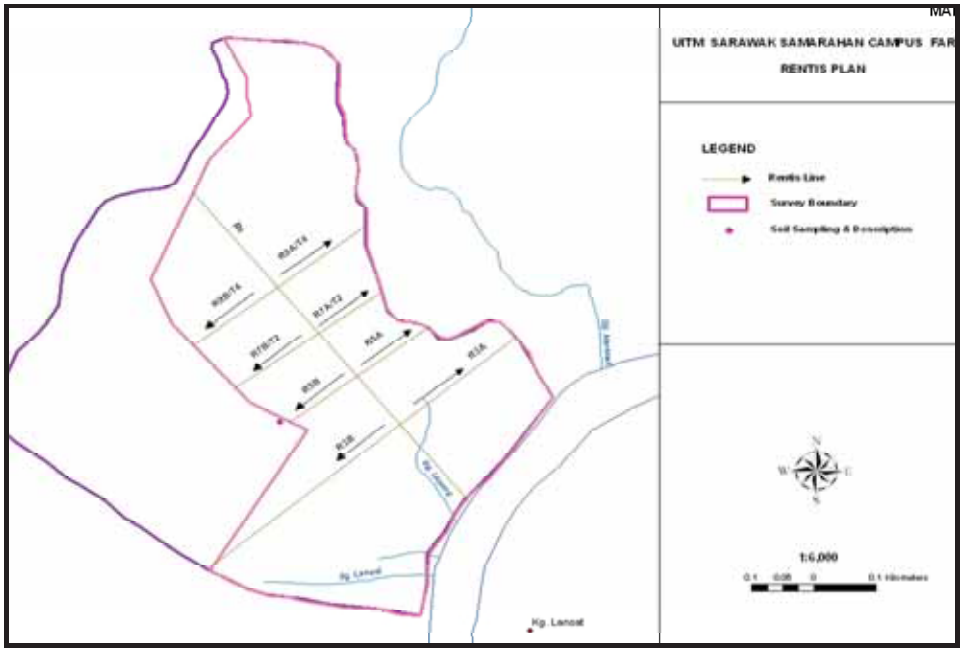

Figure 1. A Rentis Plan for the detailed soil survey at UiTM Sarawak Samarahan Campus Farm

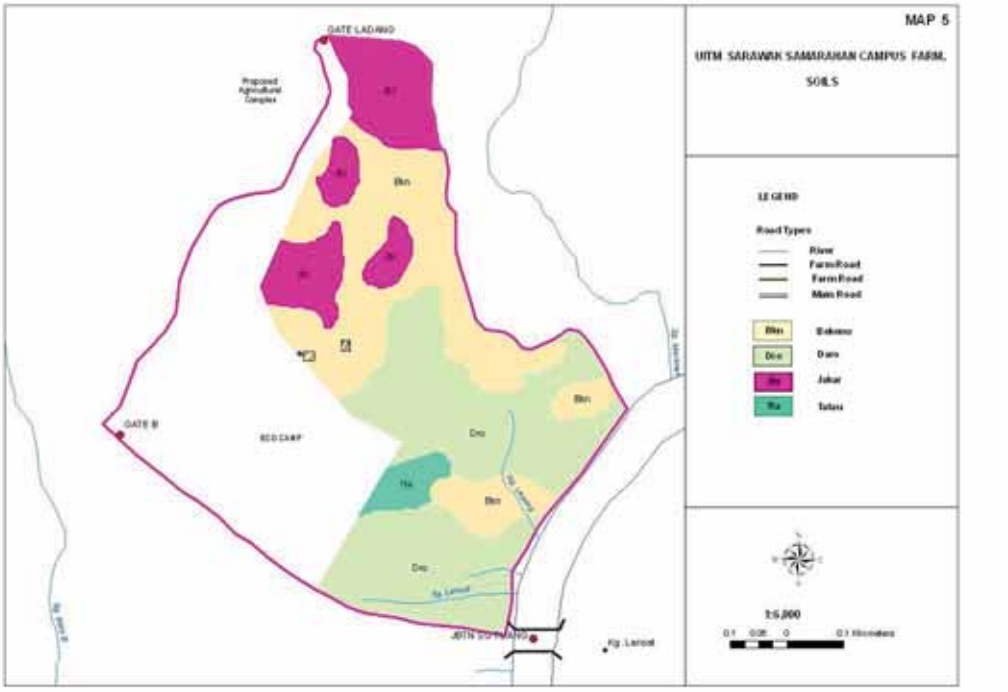

Figure 2. Soil Map of UiTM Sarawak Samarahan Campus Farm according to soil series

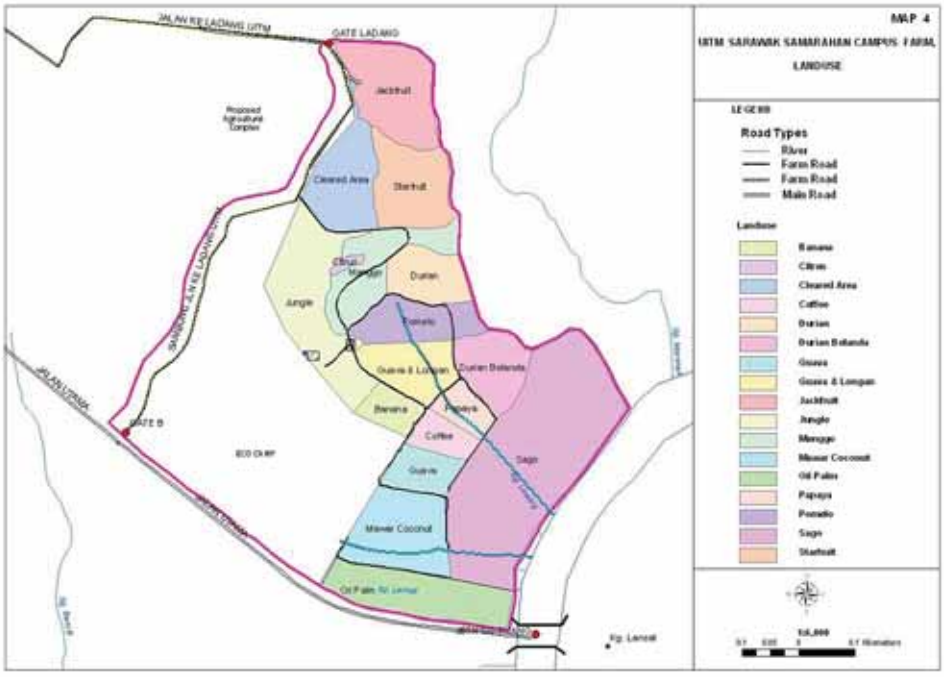

Figure 3. Present Crop plot of UiTM Sarawak Samarahan Campus Farm

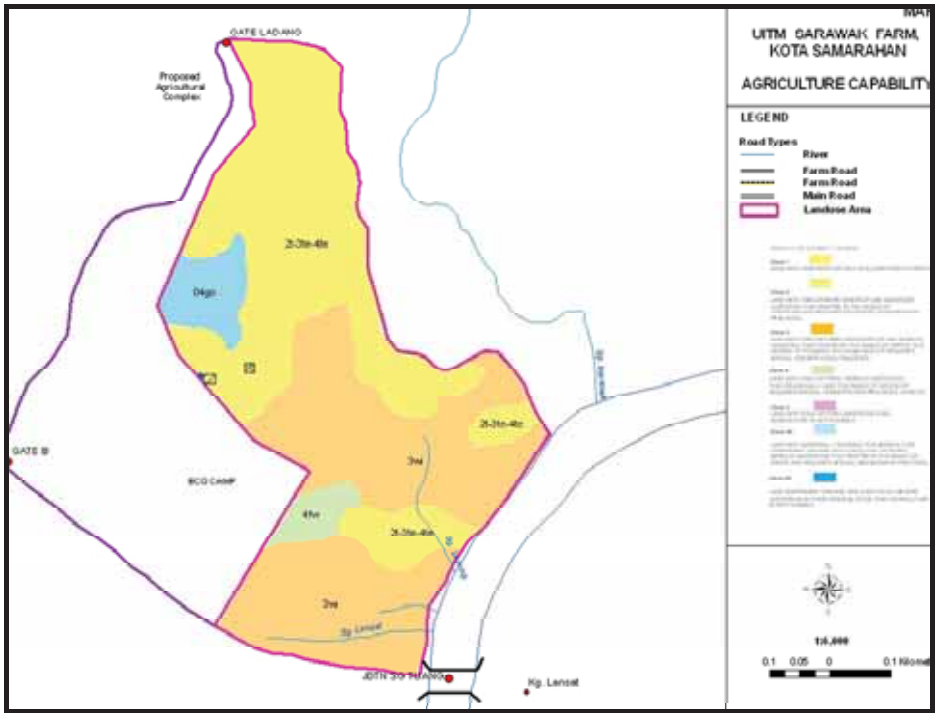

Figure 4. Agriculture Capability of the UiTM Sarawak Samarahan Campus farm.

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Measurement of Dielectric Behavior of Fertilized Soil at Microwave Frequency

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Abstract

The paper presents the experimental results which have been carried out for understanding the behavior of solid dielectrics in the form of soil at microwave frequency and check the dielectric and electric response of some fertilizers in soil in presence of microwave energy. A simple and rapid measurement method (two point method) was used to determine the dielectric constant at microwave frequency. The microwave beam generated by microwave source was reflected by the dielectric sample which was placed in rectangular waveguide and fundamental mode was considered. The microwave source reflection klystron of frequency range of X- band was used. The soil samples were prepared by mixing different concentrations of some fertilizers in soil. The soils were taken from 'Behror Alwar (RAJ)' and different fertilizers 'Urea, Shree Ram-33, Shree Ram-50P, D.A.P., Mosaic' were taken from market.

Keywords: Dielectric constant, Dielectric loss, a.c. conductivity, Relaxation time, Reflection, Two point method

1. Introduction

The behavior of dielectric materials in presence of electromagnetic (EM) field is entirely different from that in presence of direct current field. In order to understand the behavior of dielectric materials under the action of electromagnetic fields, one has to investigate its interaction in such EM field. In the presence of an alternative electric field, the dielectric materials get polarized along the field direction. The degree of polarization depends upon the applied EM power and the nature of material itself (1). The static dielectric constant and dipole moment values are the measure of the polarization of the material at low frequencies. However, presence of high frequency field, there exist a time lag in the attainment of equilibrium in system with the changing field and hence an anomalous dispersion (dielectric constant decrease within increase in frequency) takes place. This in turn gives rise to the dielectric relaxation. Spatial and temporal variation of the moisture content in the surface layer of soil is considered important in agriculture. Its knowledge is important for the sowing, development, successful maturation of a crop along with rainfall runoff prediction agricultural yields forecasting and boundary layer heat exchange for meteorological and climates studies(2). The different percentage of fertilizer content in the soil gives rise to a large variation in the dielectric constant. Thus, the knowledge of the variation of dielectric constant of the soil at different fertilizer content is necessary for the efficient use of soil.

1.1 Role of porosity in soil fertility

Porosity of the soil greatly helps to judge the moisture movement within the soil. Macro pores allow readily movement of air and water. It does not hold water under normal condition. In contrast, macro pores can hold more water and restrict the movement of air and water in soil. Thus, in sandy soil, inspite of the low total porosity, the movement of air and water is surprisingly rapid because of the dominance of the macro pore spaces. Porosity of soil is easily changed. Any operation that reduces aggregation and decreases the amount of organic matter in the soil, decreases pore space(3). In the present work, dielectric constants of the loamy sand soils with different concentration of different fertilizers content and fixed moisture content were measured at fixed frequency 9.8 GHz in the laboratory condition.

2. Material and method

The two-point method of measuring dielectric constant is used for the measurements of dielectric properties. An X-band microwave bench operating at 9.8 GHz in the TE10 mode with slotted section was used and the shift of minima is needed

in this technique. The soil samples were prepared by mixing different concentrations of some fertilizers in soil. The soils were taken from 'Behror, Alwar (RAJ)' and different fertilizers 'Urea, Shree Ram-33, Shree Ram-50P, D.A.P., Mosaic' were taken from market. First, the soil sample was oven dried and then weighed. Then measured quantity of water (1.5ml) and different quantity (according to agriculture department) of different fertilizers was added and allowed four hours to facilitate internal drainage, subsequent homogenous mixing and settlement. The dielectric constant (ε') and dielectric loss (ε") of the samples were measured after determining the weight of the sample at every reading.

From the measurement of dielectric constant and dielectric loss, other electric parameters as a.c. conductivity (σ), and relaxation time (τ) can be obtained (4).

$$\sigma = \omega\varepsilon_0\varepsilon''$$

And

$$\tau = \varepsilon''/\omega\varepsilon'$$

Where

ω is angular frequency, f = 9.8 GHz

ε_0 is permittivity of free space.

3. Results and Discussion

3.1 Dielectric constant variation

The variation in values of dielectric constant and dielectric loss with percentage of fertilizers content are measured and plotted in fig.1,2,3,4 and 5, Similarly, the a.c. electric conductivity and relaxation time with variation of percentage fertilizers content are plotted in fig.6,7,8,9 and10. By measurements it is observed that there is a very little variation in the dielectric constant of soil with fertilizers. Another feature is that the higher fertilizer contents have higher dielectric constant and higher dielectric loss in soil as compared to that low fertilizer content at a given frequency. The value of dielectric constant (ε') and dielectric loss **(**ε") increases with fertilizer contents. This slow increase is due to the presence of fertilizers. When the polar molecules are very large, then in presence of high frequency electromagnetic field, the rotary motion of polar molecules is not sufficiently rapid to attain the equilibrium with the field. Therefore, the displacement current acquires conductance dissipation energy. Thus, the a.c. conductivity is increase with dielectric loss. The relaxation time is increase due to increasing hindrance to the process of polarization.

3.2 Fertility variation

The fertilizers increase the pore space of the soil (3). Due to more pore space the dielectric constant (ε') increases and the fertility of soil is also increased. The different types of fertilizers have different organic components. The soil is also affected by these different organic components. Fig.11, shows comparative increasing in dielectric constant (ε') of different types of fertilizers. According to this graph, the dielectric constant of soil with SHREE RAM-33 is more than other and it increases speedily as compared to others. The dielectric constant of soil with D.A.P. is less than others and it increases slowly as compare to others. Therefore, the above experimental results clearly show that SHREE RAM -33 speedily increases the pore space of soil as compared to other fertilizers.

4. Conclusion

Study of the properties of dry and fertilized sand soil at microwave frequencies in the laboratory is useful in Agriculture. The good results are obtained for the Dielectric Constant, dielectric loss, a.c. conductivity and relaxation time of different samples at X-band frequencies. The a.c. electrical conductivity and relaxation time depend upon the dielectric loss, which represents attenuation and dispersion. The dielectric properties of the solid dielectrics in the form of fertilized soil are useful in understanding the structural behavior of soil. These studies are also useful for increase the fertility of soil. The result of such studies may not be only important for the understanding of the fundamental nature of the response of the particulate soil to the high frequency electromagnetic fields but also of applied nature, useful to increase the pore space of soil.

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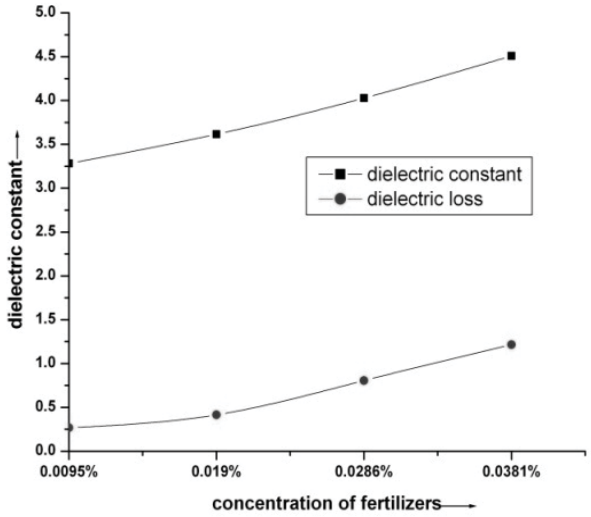

Figure 1. Variation of dielectric constant and dielectric loss with fertilizer content for SHREE RAM-33

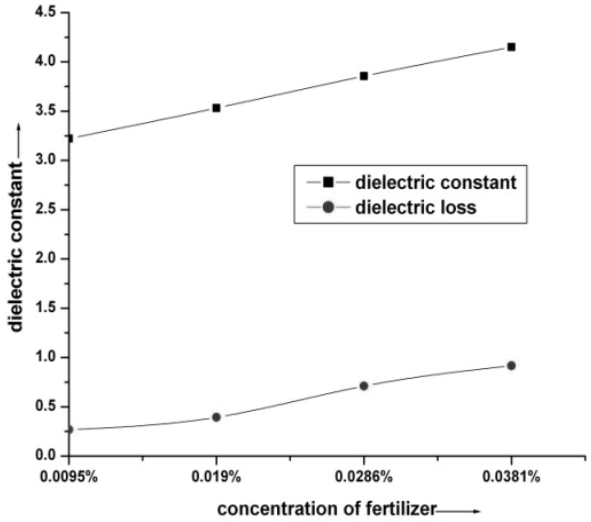

Figure 2. Variation of dielectric constant and dielectric loss with fertilizer content for SHREE RAM-50P

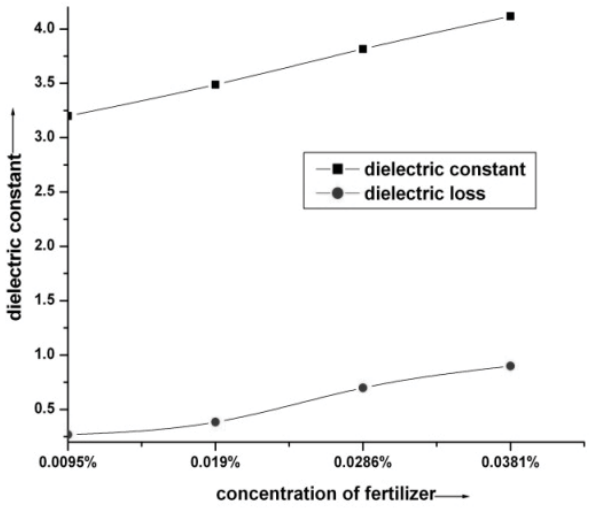

Figure 3. Variation of dielectric constant and dielectric loss with fertilizer content for MOSAIC

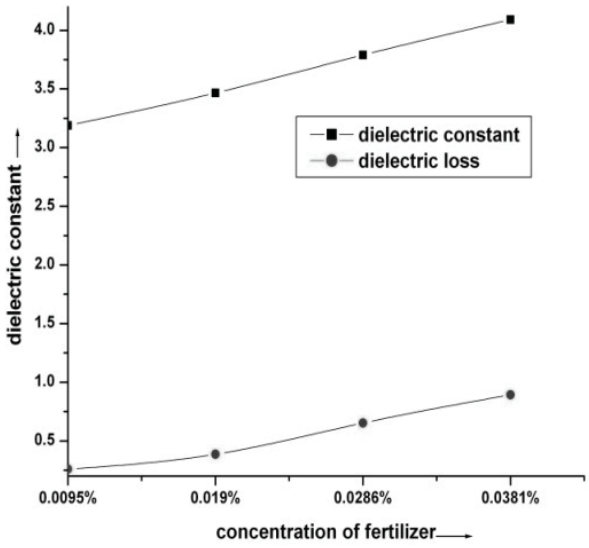

Figure 4. Variation of dielectric constant and dielectric loss with fertilizer content for UREA

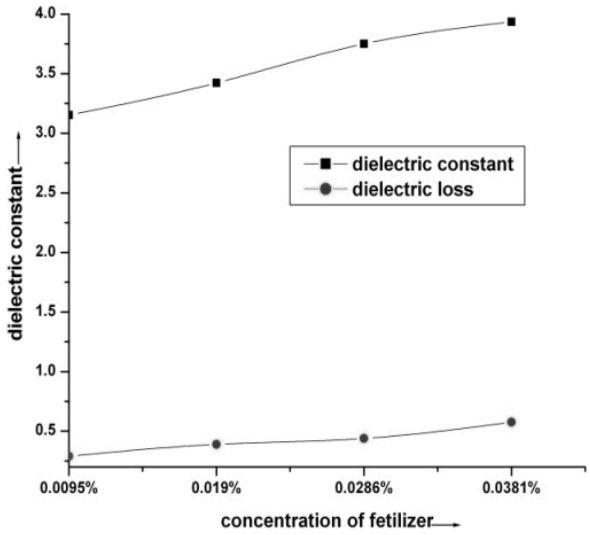

Figure 5. Variation of dielectric constant and dielectric loss with fertilizer content for D.A.P

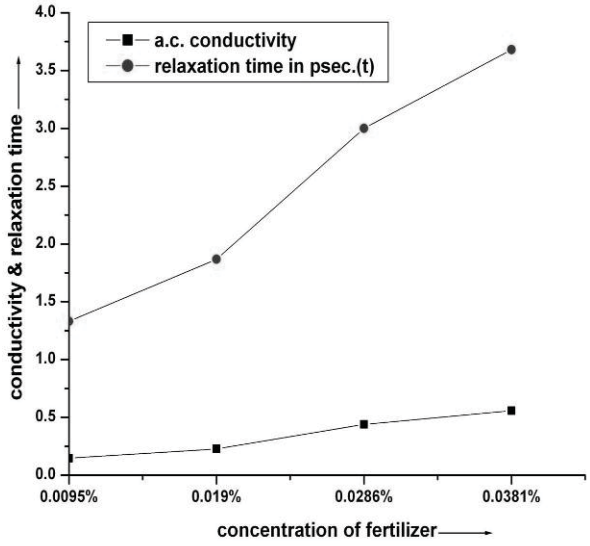

Figure 6. Variation of a.c. conductivity & relaxation time with fertilizer content for SHREE RAM-33

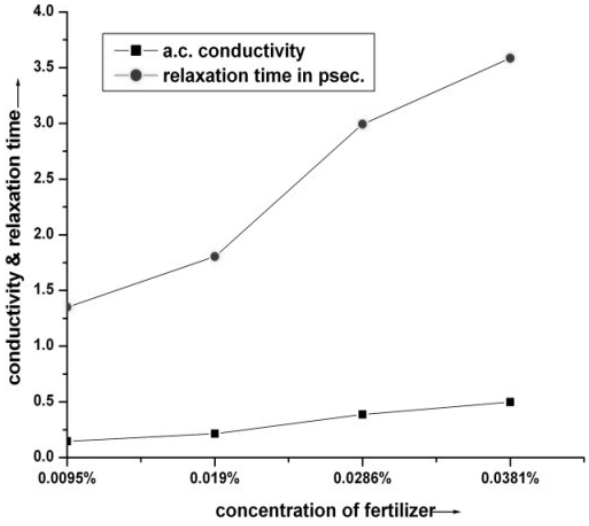

Figure 7. Variation of a.c. conductivity & relaxation time with fertilizer content for SHREE RAM-50P

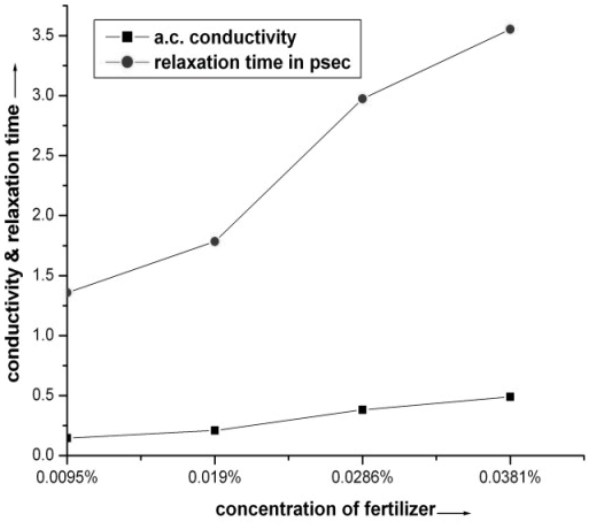

Figure 8. Variation of a.c. conductivity & relaxation time with fertilizer content for MOSAIC

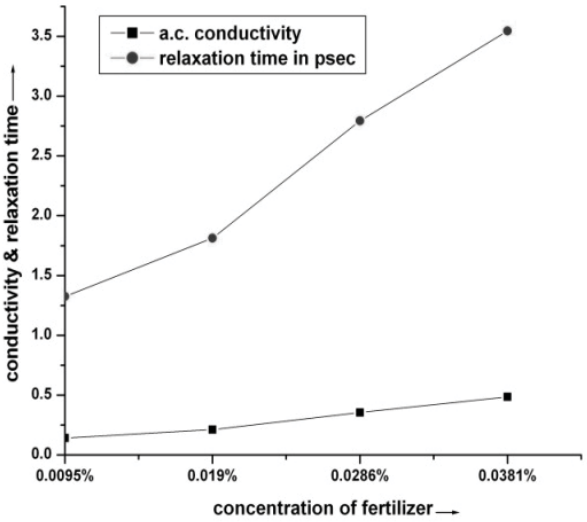

Figure 9. Variation of a.c. conductivity & relaxation time with fertilizer content for UREA

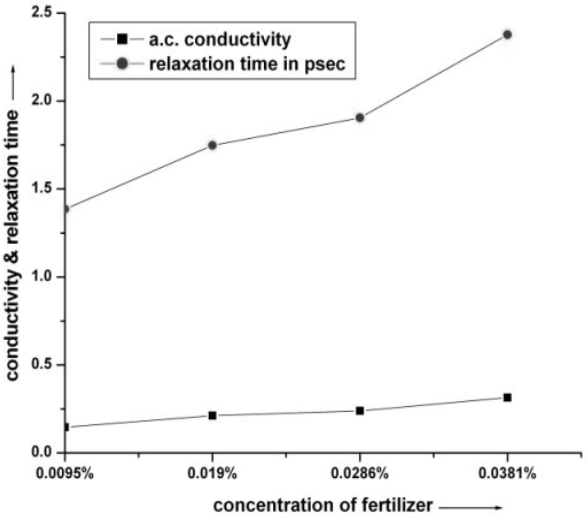

Figure 10. Variation of a.c. conductivity & relaxation time with fertilizer content for D.A.P

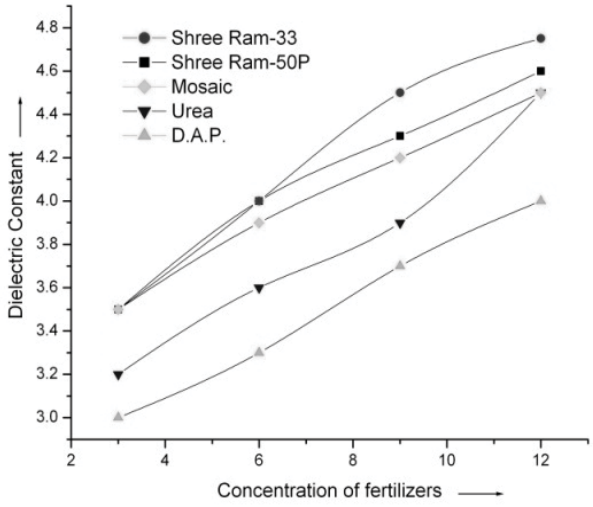

Figure 11. Graph between dielectric constant and concentration of fertilizers

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Studies on the Responses of Root, Shoot and Drought Resistance in the Seedlings of Forage Triticale to Water Stress

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Abstract

In order to investigate the identification index of drought resistance in the forage triticale seedlings, 30 triticales with different genotypes were identified by 20%–PEG6000. Results showed that responses of each gene to water stress were relatively obvious, water content decreased, the root-shoot ratio enhanced, and leaf curvature and root hyperplasied; There was significant difference among different genotypes, material 27,29,30 showed a good representation to water stress, and their root status were also good. The variation regularity of water content and root hyperplasia was good and they had a great significant correlation. But the variation of root-shoot ratio is relatively complex, other factors should be taken further consideration in the application.

Keywords: Water stress, Water content, Root-shoot ratio, Leaf curvature, Root hyperplasia

1. Introduction

Drought is a worldwide problem, and only 0.007% freshwater resources in the world surface could be utilized by human beings. However, the distribution of these limited freshwater is greatly uneven in the world. There are 100 countries or regions which is facing the problem of water deficit, among which 28 countries were listed the topest country under sever deficit (Zhang, 2003). Consequently, studies on the drought resistance of crops are a focus in the agriculture science at present. Triticale is a hybrid of wheat (Triticum) and rye (Secale), and a new cultivar first bred by chromosome engineering breeding technology.Triticale, as a cultivated crops with mass production and economic values first appeared in 1969(Zillinsky, 1974, P. 375). It possesses not only the properties of high production and good quality, but also high resistance to environmental condition of abiotic stress, such as drought and so on. It is abroad suitable to plant in the cropland with bad geological conditions which is not suitable for major economic crops. Investigation of the growth of root and shoot and the variation of water content in triticale under water stress, would not only be important for the screening and identifying excellent drought resistance clones of triticale, but also offer references to the studies on drought resistance of other crops. Therefore, 30 materials with different genotypes were undertaken osmotic stress trial by using 20%–PEG6000 in the present paper. Water content, root-shoot ratio, leaf curvature and root hyperplasia of the tested materials were observed, and the growth of root and shoot and the variation of water content in triticale under water stress were undertaken comprehensive investigation.

2. Materials and methods

2.1 Experimental materials

30 advanced lines with different drought resistance bred by wheat crops breeding team of in school of agriculture, Shihezi University were listed in Table 1 in detail, and were cultured in light culture room of Lvzhou ecological laboratory.

2.2 Material treatment and experiment design

Plump seeds without diseases were selected, sterilized by 70% ethanol for 3min, and washed by deionized water. Seeds were cultured in 10cm petri dishes to germinate. Small seedlings were divided into two groups, namely control and stress group until one leaf and a bud, and then transplanted into Hoagland's solution. Seedlings were undertaken water culture with light and ventilation until 3 leaves.

Due to the fast growth rate in triticale seedlings under normal culture, in order to eliminate growth impact on the experiment results, all indices experiment required were determined before stress, and defined as "0h". Then seedlings in stress group were treated by 20%–PEG6000, and those in control group were still undertaken water culture. Indices were determined after 72h, and stress and control group were defined as "72hCK" and "72hWS", respectively. Each

index with three replicates, each replicate with 5 seedlings.

2.3 Determination content of materials and data processing

2.3.1 Determination of water content

Both aerial and underground part of 72hCK or 72hWS seedlings at 0h was weighed. Fresh weight (FW) and dry wight (DW) dried until constant weight was determined. Water amounts were obtained by the calculation of the difference between FW and DW. The ratio of water content to FW was material's WC (water content), including leaf and root water content (LWC and RWC). Water loss rate (WL) after stress was calculated using WC, including leaf and root water loss rate (LWL and RWL), and then correlated responses of WC to water stress were investigated.

$$WC= [(FW-DW)/FW]\cdot 100\%$$

$$WL=\text{non stressed } WC\cdot(1-\text{stressed } WC)$$

2.3.2 Determination of root-shoot ratio

Both aerial and underground parts of 72hCK or 72hWS seedlings at 0h were weighed. FW and DW dried until constant weight were determined. The ratio of FW or DW of aerial part to underground part was fresh or dry root-shoot ratio, respectively. Correlated responses of root-shoot ratio to water stress were investigated.

Root-shoot ratio(R/T) =Root weight/leaf weight

2.3.3 Determination of leaf curvature under 72h stress

For leaves in the seedlings of three leaves were quite tender, according to the results of leave curvature by visual observation, with reference to classification methods of leave curvature introduced by O'Toole et al (1980, PP. 428-432)and Zhang(1998, PP. 608-612), tested leaves were classified into five curvature grades as follows:

1: flat-unfolded

2: moderately asymmetrical with arcs

3: half-rolled

4: curly

5: curly, like bucket

2.3.4 Determination of root hyperplasia under 72h stress

Triticale is a plant with quite high drought resistance, and active stress response occurring in the root system of materials was quite obvious under water stress, such as the enlargement of root system, the increment of lateral roots and so on. In our observations, under the stress of high osmotic pressure, it was noted that several materials excluding the enlargement and increment of lateral roots, could result in adventitious roots in the stems during the stress period of 24h to 48h, not similar to those under non stress. Compared to general new roots, adventitious roots possessed the features of wide diameter, long root hair zone and so on. According to the extent of root hyperplasia and the quantity of the observed new roots, root hyperplasia was classified into five grades as follows:

1: no new adventitious roots in basal, no enlargement of root system, no increment of lateral roots, and obvious water loss in root system.

2: no new adventitious roots in basal, no enlargement of root system, and no increment of lateral roots.

3: 0-1 new adventitious roots in basal, visible enlargement of root system and increment of lateral roots.

4: 1-2 new adventitious roots in basal, visible enlargement of root system and increment of lateral roots.

5: more than 2 adventitious roots in basal, visible enlargement of root system and great increment of lateral roots.

3. Results and analysis

3.1 Effects of water stress on LWC and RWC

In the studies of plants drought resistance, WC was frequently used as a drought resistance index of crops. As seen from Figure 1, without stress treatment, WC difference among different materials was relatively low. Average LWC and RWC in 72hCK and WC in materials varied insignificantly compared to 0h. However, both LWC and RWC in 72hWS treated by 20% PEG6000 decreased significantly, compared to those under no stress, and WC difference among materials increased significantly. Water loss of leaves was grossly larger than that of roots.

It could be also noted that, during the seedlings of three leaves, under the same treatment, RWC was all higher than LWC, and LWL in most materials was higher than RWL after stress treatment (Table 2), which probably ascribed to the triticale's physiological structure and osmotic pressure in such growth period.

Variance analysis indicated that LWL or RWL in different materials varied significantly (Table 2), namely that

responses of water condition among different materials to water stress were significantly different. Plant WC after stress treatment was quite higher, material 28, 30 with lower WC had less water loss, while material1, 4 had more water loss.

3.2 Effects of water stress on root-shoot ratio

As known from the previous conclusion that during the seedlings of three leaves RWC was all higher than LWC, and LWL in most materials was higher than RWL after stress treatment, fresh root-shoot ratio of triticales in the seedlings was higher than dry root-shoot ratio, and such ratio was susceptible to water variation of plants, which was in agreement with practical results of our experiment. Consequently, from the angle of substance accumulation, for the seek of analysing growth status of root and shoot, fresh root-shoot ratio was more proper.

As seen from Figure 2 and Table 3, after 72h water stress, excluding individual material, root-shoot ration in most materials increased significantly more than that in 72hCK. Compared to 0h, increment of root-shoot ratio in 72hWS was less significant than in 72hCK, even reduction in individual material. Compared with 0h and 72hCK without stress treatment, excluding individual material, the later showed a decline trend compared to the former, which conformed to the previous study (Chen, 2004, PP. 574-578). Therefore, we could speculate that significantly more increment of root-shoot ratio in 72hWS than in 72hCK didn't necessarily ascribed to the increasing of root system stimulated by stress, but presumably because the growth of triticles was inhibited by water stress compared to the normal water condition.

Variance analysis of different materials' root-shoot ratio under different treatment indicated that difference existed among materials at each treatment, but different treatment failed to induce significantly regular variation of root-shoot ratio differences among materials (Figure2, Table 3). Correlation analysis of root-shoot ratio after stress to WC or WL indicated that there was an insignificantly negative correlation between root-shoot ratio and WC, and an insignificantly positive correlation between root-shoot ratio and WL(Table 6), which was not in line with our prediction. Compared with Table 3 and 2, we could speculate that material with higher root-shoot ratio after stress did not necessarily have a good water condition.

3.3 Investigation of leaf curvature and its relationship with water after stress

Several studies have indicated that leaf curvature resulted from the reduction of leaf cell turgor. Leaf curvature used as an index of crops drought resistance have been applied in the breeding and planting (O'Toole, 1980, PP. 428-432; Neil, 1986, PP.257-271). Currently, reports on the leaf curvature of wheat crops in the seedlings were less available, while studies about that in mature period were quite common. Studies on the wheat during flowering stage by Zhang et al(1998, PP. 608-612) indicated that leaf curvature of several materials was large with higher water potential, while leaves of some materials with lower water potential was flat-unfolded.

In the present paper, leave curvature of several materials varied greatly after stress, but leaf curvature of materials with low WC was generally large(Table2, 4). According to the correlation analysis, there was a great significantly negative correlation between leaf curvature and LWC, and a great significantly positive correlation between leaf curvature and WL(Table 6), which was different from the relationship between leaf curvature and drought resistance in mature period. According to the material characteristics in the seedlings of three leaves, due to its tender tissues and high WC, impact of LWC on leave curvature was stronger in the seedlings than in growth or mature period. Accordingly, when leave curvature was applied to investigate the drought resistance of materials, characteristics of growth period should be considered, and materials in the seedlings should be treated differently from those in mature period.

3.4 Investigation of root hyperplasia and its relationship with water and root-shoot ratio after stress

Under water stress, there were new adventitious roots among most materials. Their root system got longer, and lateral roots increased. Especially during the stress period from 24h to 48h, new adventitious roots different from new roots under normal water condition appeared the most directly. Compared to the normal new roots, such roots possessed the characteristics of wide diameter, long root hair zone and so on. Taken all previous characteristics together, root hyperplasia of each material was evaluated.

Results showed that materials with root hyperplasia showed good water and growth status after stress (Table2, 5). Correlation analysis indicated that there was a great significantly positive correlation between root hyperplasia and WC, and a great significantly negative correlation between root hyperplasia and WL after stress (Table 6). Therefore, we could speculate that root hyperplasia was of great positive significance to plant's water adsorption.

Correlation analysis indicated that there was an insignificantly negative correlation between root hyperplasia and dry root-shoot ratio after stress (Table 6), which was not in line with the deduction that increment of root-shoot ratio after stress resulted from the stimulation of root system. With reference to the previous results of root-shoot ratio observation, it was noted that increment of root-shoot ratio of several materials didn't ascrib to the increasing of root system, but growth inhibition of the aerial part, even damages to membrane and substance loss induced by strong water stress, which we should pay attention to when we used root-shoot ratio to evaluate drought resistance of materials in the

seedlings.

3.5 Cluster analysis of water condition and root hyperplasia among materials under stress

According to our upper analysis, characteristics of RWC, LWC and root hyperplasia after stress, which were regular and reliable in the experiment, were subjected to UPGMA cluster analysis. First, values of each index should be standardized (Figure 3).

The 30 materials could be grouped into 2 main groups. Group 1 contained 9 materials, i.e. 1, 2, 3, 4, 5, 7, 8, 9, 10. All of them showed a bad water and root condition under water stress; other materials belong to Group 2. All of them showed a good water and root condition under water stress. In group 2, materials with different water and root level could be grouped into several subgroups. Material 27, 29 and 30 showed the best water and root condition, while 1, 4 showed the most sensitivity.

4. Discussions

There are many factors affecting the drought resistance of crops, and these factors are not independent, but interact and affect with one another. Therefore, in order to investigate crop drought resistance, attention should not only be paid to identifying representative typical characteristics, but also to their relationship and interaction of those characteristics.

In the present study, results showed that triticale WC varied significantly under stress, and significant difference existed among materials with certain rules, which was easy to investigate and analysis. Meanwhile, active responses of root system to water stress occurred, i.e., lateral roots increased, root system enlarged and new roots grew. The significant correlation between root hyperplasia and WC confirmed that strong mutual impacts existed between water and drought resistance characteristics of root system, positive responses of root system to water stress had a good water condition, and conversely, good water condition in plants could guarantee substance synthesis and promote the growth of root system. All those indicated that possible properties materials with strong drought resistance possessed were of some reference significance for studies on drought resistance of triticales in the seedlings.

Investigations of leaf curvature indicated that there was a significant correlation between leaf curvature and WC, which suggested that curvature variation of tender materials in the seedlings showed a stronger independence to water. Due to the difference from the investigations of leaf curvature in mature period, leaf curvature used as an index for drought resistance, we should fully consider the characteristics of materials in the seedlings.

Investigations of root-shoot ratio indicated that materials with larger or obvious increment of root-shoot ratio didn't show an active impact on their water condition, and results of correlation analysis were not significant. In addition, there was no insignificantly positive correlation between root-shoot ratio and root hyperplasia, which was very different from theory. Consequently, we could speculate that strong water stress could make some materials with less drought resistance growth in aerial part inhibited, damages to membrane, substance loss, and thus root-shoot ratio increased. In view of the complexity of factors affecting root-shoot ratio, when it was applied to identify the drought resistance of triticales, possible factors should be considered, other relative characteristics should be taken into comprehensive consideration and some proper rules should be explored.

Taken together, material 27, 29,30 showed a good water and root condition, while 1,4 showed a bad condition. All these materials should be considered to further application in the following breeding practice about drought resistance.

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Table 1. Triticale material directory

Serial number	Material	Serial number	Material	Serial number	Material
1	04 Spring H147-148	11	04Spring H159-160	21	04Spring H431-432
2	04Spring H21-22	12	04Spring H855-856	22	04Spring H507-508
3	04Spring H799-800	13	04Spring H631-632	23	04Spring H193-194
4	04Spring H513-514	14	04Spring H127-128	24	04Spring H567-568
5	04Spring H939-940	15	04Spring H613-614	25	H04-7
6	04Spring H331-332	16	04Spring H13-14	26	04Spring H499-500
7	04Spring H729-730	17	04Spring H365-366	27	04Spring H447-448
8	04Spring H699-700	18	04Spring H757-758	28	04Spring H547-548
9	04Spring H851-852	19	04Spring H299-300	29	04Spring H861-862
10	04Spring H415-416	20	04Spring H499-500	30	H05-5

Table 2. LWL and RWL of different materials under water stress and variance analysis

Mat erial	RWL after stress compared to 72hCK	LWL after stress compared to 72hCK	Mat erial	RWL after stress compared to 72hCK	LWL after stress compared to 72hCK
1	0.119±0.003 a	0.162±0.006 ab	16	0.095±0.003 ghij	0.121±0.010 ijkl
2	0.103±0.002 def	0.147±0.003 def	17	0.099±0.002 fghi	0.124±0.006 ijk
3	0.104±0.002 cdef	0.153±0.012 bcd	18	0.090±0.002 jk	0.129±0.005 ghij
4	0.119±0.011 a	0.169±0.002 a	19	0.092±0.005 ijk	0.126±0.014 hij
5	0.107±0.001 bcde	0.154±0.008 bcd	20	0.089±0.003 jk	0.127±0.010 hij
6	0.098±0.005 fghi	0.155±0.005 bcd	21	0.090±0.000 jk	0.133±0.008 ghi
7	0.105±0.002 cdef	0.162±0.007 ab	22	0.093±0.003 hijk	0.113±0.005 kl
8	0.107±0.004 bcde	0.152±0.007 bcd	23	0.100±0.004 efgh	0.112±0.007 kl
9	0.111±0.005 bc	0.149±0.008 cde	24	0.086±0.006 kl	0.113±0.003 kl
10	0.110±0.002 bcd	0.160±0.008 abc	25	0.086±0.005 kl	0.124±0.004 ijk
11	0.101±0.001 efg	0.146±0.007 def	26	0.085±0.005 kl	0.117±0.001 jkl
12	0.098±0.007 fghi	0.139±0.005 efg	27	0.082±0.006 l	0.113±0.001 kl
13	0.113±0.005 ab	0.155±0.004 bcd	28	0.080±0.012 l	0.139±0.012 efg
14	0.104±0.005 cdef	0.137±0.007 fgh	29	0.086±0.003 kl	0.129±0.005 ghij
15	0.102±0.002 efg	0.130±0.008 ghi	30	0.080±0.002 l	0.110±0.007 l

Different letters after data in the same column in table showed significant difference at 5% level.

Table 3. Data, variance analysis and variation of root-shoot ratio under water stress

Material	0h dry root-shoot ratio	72hCK dry root-shoot ratio	72h dry root-shoot ratio	Material	0h dry root-shoot ratio	72hCK dry root-shoot ratio	72h dry root-shoot ratio
1	0.259±0.002 hijk	0.236±0.021 hijklm	0.323±0.006 efgh	16	0.309±0.009 defg	0.278±0.007 cdefgh	0.306±0.059 efghi
2	0.319±0.025 def	0.320±0.029 abc	0.418±0.019 a	17	0.325±0.008 de	0.274±0.004 defghi	0.300±0.025 ghi
3	0.323±0.027 def	0.332±0.027 ab	0.398±0.018 ab	18	0.261±0.020 hijk	0.227±0.015 jklm	0.307±0.037 efghi
4	0.347±0.017 bcd	0.332±0.004 ab	0.372±0.039 bcd	19	0.324±0.018 de	0.261±0.034 fghijk	0.336±0.071 defg
5	0.281±0.034 efgh	0.227±0.010 jklm	0.315±0.049 efgh	20	0.235±0.021 ijkl	0.208±0.032 mn	0.301±0.062 ghi
6	0.273±0.007 ghij	0.251±0.016 fghijklm	0.304±0.024 fghi	21	0.385±0.018 ab	0.315±0.026 abcd	0.319±0.035 efgh
7	0.388±0.010 ab	0.348±0.007 a	0.376±0.052 abcd	22	0.279±0.034 fghi	0.247±0.023 ghijklm	0.370±0.013 bcd
8	0.284±0.014 efgh	0.260±0.004 fghijk	0.301±0.026 ghi	23	0.286±0.008 efgh	0.220±0.008 klm	0.346±0.019 cdef
9	0.219±0.008 kl	0.173±0.017 n	0.265±0.054 ij	24	0.268±0.011 ghij	0.255±0.020 fghijkl	0.378±0.015 abcd
10	0.311±0.030 defg	0.235±0.009 hijklm	0.349±0.041 cde	25	0.229±0.016 jkl	0.232±0.009 ijklm	0.282±0.021 hi
11	0.376±0.031 abc	0.306±0.025 abcde	0.414±0.060 ab	26	0.281±0.043 efgh	0.266±0.035 efghij	0.322±0.024 efgh
12	0.332±0.020 cd	0.255±0.010 fghijkl	0.348±0.011 cdef	27	0.372±0.011 abc	0.350±0.020 a	0.321±0.021 efgh
13	0.313±0.038 defg	0.252±0.012 fghijklm	0.334±0.046 defg	28	0.214±0.015 l	0.211±0.031 lmn	0.295±0.027 ghi
14	0.397±0.015 a	0.294±0.026 bcdef	0.390±0.019 abc	29	0.213±0.035 l	0.225±0.024 jklm	0.221±0.011 j
15	0.353±0.036 abcd	0.286±0.028 cdefg	0.390±0.011 abc	30	0.254±0.019 hijkl	0.281±0.037 cdefg	0.319±0.043 efgh

Different letters after data in the same column in table showed significant difference at 5% level.

Table 4. Leaf curvature of 30 materials under water stress

Material	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Grade of leave curvature	5	5	5	5	4	5	5	3	4	5	5	4	4	3	3
Material	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Grade of leave curvature	3	3	1	3	3	5	5	4	3	2	1	3	3	1	1

Table 5. Root hyperplasia of 30 materials under water stress

Material	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Grade of root hyperplasia	2	2	2	2	2	2	2	2	2	2	4	2	4	4	4
Material	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Grade of root hyperplasia	4	2	3	2	3	2	3	2	3	4	4	5	4	5	5

Table 6. Correlation among different characteristics under water stress

	72hWS RWC	72hWS LWC	72hWS RWL	72hWS LWL	72hWS Dry root-shoot ratio	72hWS Leaf curvature	72h Root hyperplasia
72hWS RWC	1						
72hWS LWC	0.802**	1					
72hWS RWL	-0.998**	-0.792**	1				
72hWS LWL	-0.805**	-0.997**	0.795**	1			
72hWS Dry root-shoot ratio	-0.324	-0.173	0.316	0.176	1		
72hWS Leaf curvature	-0.637**	-0.607**	0.644**	0.616**	0.521**	1	
72hWS Root hyperplasia	0.575**	0.495**	-0.575**	-0.511**	-0.195	-0.635**	1

"*" and "**" indicated significant correlation at 5% and 1% level, respectively.

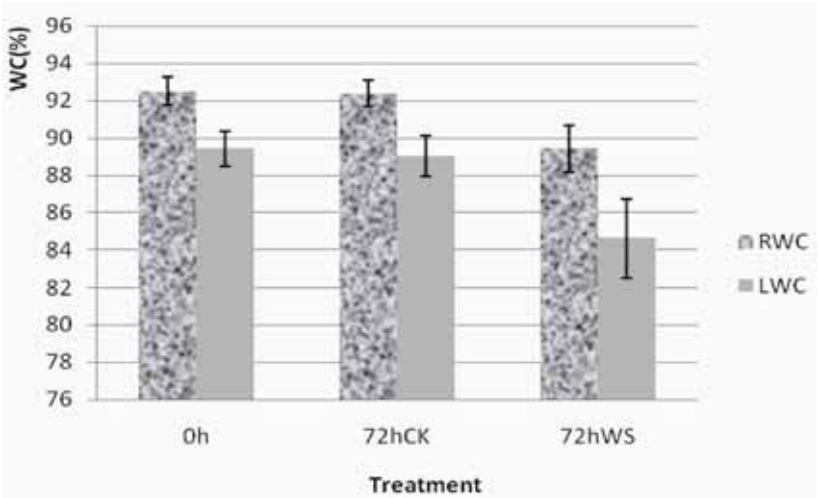

Figure 1. Effects of water stress on RWC and LWC in triticales

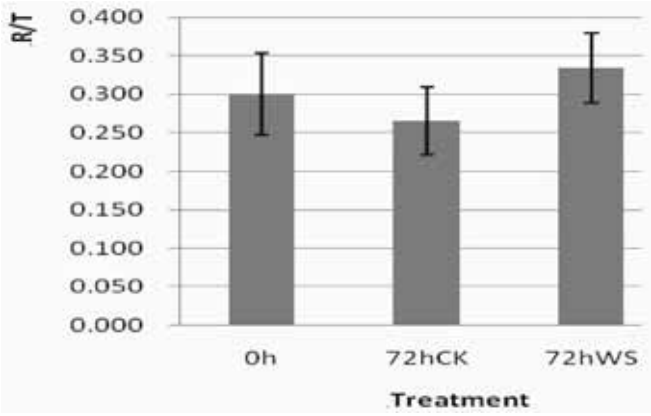

Figure 2. Effects of water stress on root-shoot ratio in triticales

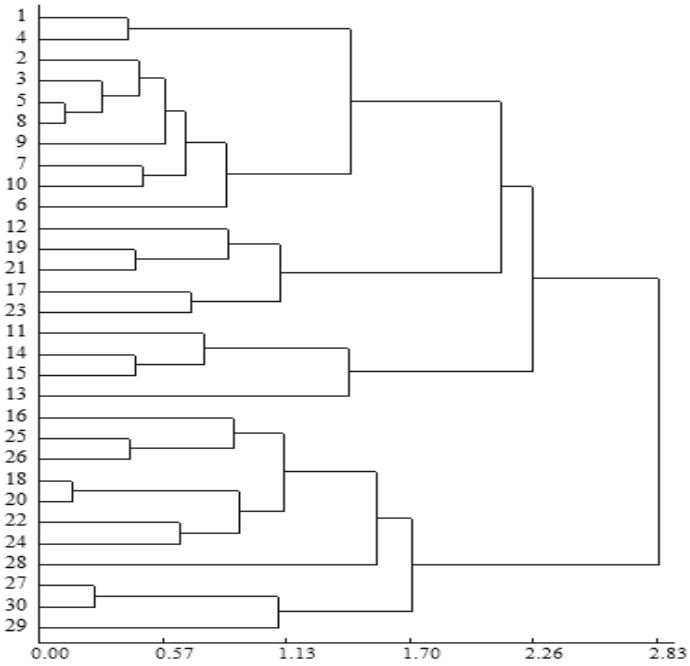

Figure 3. The phylogenetic dendrogram of WC and root hyperplasia of 30 materials using UPGMA cluster analysis

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Phosphorus Kinetics in Calves Submitted to Single Infection with *Cooperia punctata*

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Abstract

The aim of this study was to evaluate phosphorus (P) kinetics in calves submitted to a single acute infection of *Cooperia punctata*, using isotopic dilution and modelling techniques. Ten Holstein calves were used, with a mean live weight of 66.05 ± 0.30 kg. Of these, five received a single dose of 45 000 infectant (L_3) *C. punctata* larvae and the other five were maintained in a control group without infection. Twenty one days after the infection, all animals received 29.6 MBq ^{32}P by intravenous injection to evaluate P kinetics. Weight gain, feed consumption and excretion in the faeces and urine were monitored and blood was collected for seven days. After the collection period, all animals were slaughtered,

tissues collected and worms counted. The number of eggs per gram of faeces (EPG) reached 3 342 ± 194 and the number of adult worms was 12 992 ± 1 470. Final live weight, mean daily live weight gain, level of P in the plasma and its retention in control calves were higher and P excretion in the faeces less than in the infected calves. There was a negative P balance in both the control and infected calves for soft tissues and bone. A single infection by *C. punctata* negatively influenced calf performance and P kinetics, leading to lower retention of the mineral.

Keywords: Cattle, Mineral, Modelling, Parasite

1. Introduction

Cooperia sp. is one of the most prevalent nematodes of cattle in Brazil and *C. punctata* present the highest occurrence (Lima, 1998). A high level infection with this nematode may lead to nutritional deficiencies or worsen those that already exist. Vieira-Bressan et al. (1996) found a reduction in fat-free body weight, total body water and nitrogen retention in calves infected with *C. punctata*.

Despite the high prevalence of *C. Punctata*, few studies are available correlating the physiopathology of the infection (Yatsuda and Vieira-Bressan, 2000) especially those with that involve mineral metabolism. Phosphorus (P) is of particular interest due to the functions it serves in the organism, such as involvement in different metabolic activities and bone composition, among others. Its deficiency in ruminants has been widely discussed in herds kept at pasture (Underwood & Suttle, 1999).

C. punctata lives in the small intestine (duodenum and jejunum), which is also the location of highest P absorption (Schröder et al., 1995). When the parasite penetrates the intestinal wall, it causes not only diarrhea and emaciation, but can also interfere with mineral metabolism. This interference was observed with *Trichostrongylus colubriformis*, which infects the same space in the intestine of sheep (Bown et al., 1989).

Vitti et al. (2000) elaborated a model to evaluate P kinetics in goats. The P metabolism in cattle has been studied using radioisotopes by Silva Filho et al. (2000) and Vitti et al. (2001). This study aimed to evaluate P kinetics in calves infected with a single dose (45 000 infective larvae) of *Cooperia punctata*, using isotopic dilution and modelling techniques.

2. Materials and Methods

2.1 Animals and diets

Ten Holstein calves, acquired at two days of age, were kept in the Department of Preventive Veterinary Medicine and Animal Health (VPS) of the Faculty of Veterinary Medicine and Zootechny (FMVZ) of the University of São Paulo (USP) at the Pirassununga Campus, São Paulo State, Brazil. The experimental protocol was approved by the University Ethics Committee of Animal Experimentation.

The calves were bottle fed on *in natura* milk and kept in collective stalls with cement floors until weaning at two months of age. After this they received a daily ration of 1 500 g of Coast-cross (*Cynodon dactilon*) hay and 1 000 g of commercial concentrate to meet requirements defined by the National Research Council (NRC, 2001), as well as water *ad libitum* (Table 1).

2.2 Infection, radioactive and sampling method

Five calves received a single dose of 45 000 L_3 *C. punctata* larvae and five remained without infection (control). After larval innoculation the animals were housed in individual cages for metabolic study for 28 days. The adaptation period lasted 21 days and the following seven days were used for sample collection. On the 22nd day each animal was intravenously injected with 29.6 MBq of a ^{32}P solution. Blood samples (10 mL) were taken by vacutainer from the left jugular vein at 24 h intervals for seven days after the isotope administration. Blood was centrifuged and plasma separated for analysis. Trichloroacetic acid (9 mL) was added to 1mL of plasma for protein precipitation. After centrifugation (1 100 × g), inorganic P was determined by colorimetric analyses (Fiske & Subbarow, 1925).

P intake and excretion in faeces and urine were recorded for 7 days, and subsamples (10% of total outputs) were stored at 4 ºC. Faeces samples (1 g) were dried overnight (105 °C) and ashed (500 °C for 8 h). P content was determined by a colorimetric method (Sarruge & Haag, 1974). A similar procedure was used to determine P content in the consumed feed. Urine samples (30 mL) were acidified by using 100 mL of 12 N HCl, then dried (55 °C) and ashed (500 °C). Inorganic P was determined using vanadate-molybdate reagents (Sarruge & Haag, 1974). For radioactivity measurements, 1mL plasma and urine samples were added to 19 mL of distilled water in counting vials. Ashed fecal samples (1 g) were dissolved in 18 N H_2SO_4. Radioactivity of ^{32}P was measured by using Cerenkov radiation.

Specific activities in plasma and faeces were determined according to Lofgreen and Kleiber (1953). After the end of the collection period, the calves from both groups were slaughtered by 5mL.kg-1 intravenous injection of mebezonic iodine, embutramide and tetracaine cloridrate. Tissues (liver, heart, kidney, and muscles) and bone samples (12th rib) were collected from three calves from each group. The material was cleaned, weighed, and autoclaved. Samples were ground

and dissolved in 18 N H_2SO_4. The extract was transferred to vials for radioactivity determination. For P determination, bone samples were dissolved in concentrated HCl (Sarruge & Haag, 1974). Bone specific activity in 1 g DM and ^{32}P incorporation in bone were calculated according to Lofgreen & Kleiber (1953).

2.3 Parasitological Exam

Worm infection was monitored by faecal examination (eggs per gram of faeces, EPG), using the modified McMaster method (Leland, 1995). This was carried out daily until the 14th day after infection until the patency period. After this phase, faeces samples for each calf were examined weekly until the ^{32}P injection. After the injection the faeces became radioactive, and therefore were no longer used for EPG counting. Blood samples were collected weekly for determination of P, total protein and serum albumin.

At necropsy, the contents of the small intestine and mucosal scrapings were washed with running water and the volume made up to 2 L with water. After mixing, duplicate 200 mL samples (10%) were collected and preserved in 10% formalin for total worm counts and identification of larval stages. To release the worms which were adhered to the mucosa, the small intestine was incubated in distilled water at 32.8 ^{o}C for 24 h. After this period, the intestine was removed and the total contents preserved in 10% formalin for worm identification and counting.

2.4 Mathematical model

The model proposed by Vitti et al. (2000) was used for P kinetics evaluation. The representation of the kinetic model with radioisotopes is in Figure 1. The differential equations which describe the behaviour of the kinetics of the model are based on the principles of mass conservation. The system is assumed to be in partial equilibrium and the solutions for differential equations were obtained by equaling them to zero and manipulating them to obtain expressions of individual flows of interest. The symbols in Figure 1 represent the following equations:

$$F_{12} = s_1\tilde{F}_{10}/(s_2 - s_1), \quad (1)$$

$$F_{21} = \widetilde{F}_{10} + F_{12} - \widetilde{F}_{01}, \quad (2)$$

$$F_{32} = s_3 Q_3/[t(s_2 - s_3)], \quad (3)$$

$$F_{42} = s_4 Q_4/[t(s_2 - s_4)], \quad (4)$$

$$\left|F_{23} + F_{24}\right| = \tilde{F}_{02} + F_{12} + F_{32} + F_{42} - F_{21}, \quad (5)$$

$$F_{24} = (s_{3+4} - s_3)\left|F_{23} + F_{24}\right|/(s_4 - s_{3+4}), \quad (6)$$

$$F_{23} = \left|F_{23} + F_{24}\right| - F_{24}, \quad (7)$$

where, F (g/day) represents flow, s (dpm/g P) is the specific radioactivity activity on tissues, t (day) is the time from the start of the experiment until the count and Q (g) is the total content of P in a certain compartment. The notation s3+4 represents the mean specific activity in compartments 3 and 4.

The total endogenous P in the faeces was calculated as:

$$F_{e01} = F_{12}F_{01}/(F_{12}+F_{10}) = s_1F_{01}/s_2 \quad (8)$$

where F_{10} is the consumption of P and F_{01} the P in the faeces.

The true absorption of P was calculated as

$$F_{abs} = F_{10} - (F_{01} - F_{e01}). \quad (9)$$

2.5 Statistical analysis

Experimental measurements were analyzed in a completely randomized design. For flow data three calves were used to minimize manipulation of radioactive samples. A comparison of means between each treatment was carried out using the General Linear Model (GLM) procedure (SAS, 2000). Treatment means were assessed using the least significant difference method when overall treatment effects were $P<0.10$.

3. Results and discussion

The infected calves showed softened faeces, and two animals manifested diarrhea with a more liquid faeces, as well as depression and teeth grinding, suggesting abdominal discomfort and/or a painful process. The mean EPG counts from week 0, 1, 2, 3 and 4 after infection were: 0, 0, 80 ± 20, 2 000 ± 398 and 3 342 ± 435. The mean of total adult *C. punctata* number was 12 992 ± 1 470 and of immature forms were 289 ± 76. According to Ueno & Gonçalves (1998), an infection by *Cooperia* sp. is considered severe when animals show an EPG and parasite number greater than 200 and 10 000 respectively. Despite the higher EPG (3 342) and worm burden (12 992) the calves here showed only softened faeces, which were runny in only two animals, soon after infection, and these animals recovered in a few days, indicating a moderate infection. This was confirmed by the values of the total protein and albumin, which still in the normal range.

Mean values of dry matter (DM) and P intake, performance, plasmatic (total protein, albumin and P), as well as variables related to P metabolism, for control and infected groups, are shown in Table 2. Final live weights, mean daily weight gain, P level in plasma and P retention in the control group were higher than the infected group. P excretion in faeces was higher in the infected group. No significant differences were found between groups for DM and P intake, total protein, albumin, endogenous P in faeces and biological availability. No reduction in feed intake was observed in this study, even though this is one of the symptoms associated with infection by *Cooperia* sp. (Armour et al., 1987). This indicates that the effects found on P metabolism are directly related to the influence of the parasites on the nutrients in the diet. The control calves showed higher weight gains, P levels in the plasma and its retention, with lower P excretion in the faeces. This negative effect on utilization of the diet and in particular of P utilization due to an acute infection of C. *punctata* is therefore evident.

For both treatments, means were within the normal range for the plasma P (4 to 8 mg.100 mL^{-1}) for young bovines (Rosemberger, 1979), but lower values were observed in infected calves. This decrease was also reported by Coop & Field (1983), who observed a fall in plasma P in lambs experimentally infected by *T. vitrinus*, from the 8^{th} week of infection. Oliveira (2000) observed a marked decrease in plasmatic P of calves experimentally infected with 150 000 L_3 *C. punctata*, a higher number than used in the present study. Such losses may be related to the plasma flow through mucosal injuries caused by parasites, reported by authors that have studied *T. columbriformis* infections (Holmes, 1985; Poppi et al., 1986).

The biological availability values were high for both treatments (control = 85.54% and infected = 76.53%), being above the 70% level indicated by the NRC (2001). This uses the premise that biological availability is a trait associated with the feed, not taking into consideration the interaction of the animal with this variable, thereby using estimates.

It was seen that the P metabolism in young animals was directed towards ensuring growth, indicating the need for an efficient system of mineral absorption. As a high availability of P was observed, the value of P excreted in the faeces was on average 43.93 and 30.17% of the total consumed for the control and infected treatments, respectively. These values are low if compared to literature values. Silva Filho et al. (2000) showed that excreted P in the faeces in ruminants corresponded to 70% of consumed P. The P excreted in the faeces of infected animals have was approximately 1.7 times higher than P in the faeces of control animals, again indicating the interference of *C. punctata* in the use of this mineral.

Retention is the best indicator of mineral use. For control calves this was 8.68 g P/day, compared with 6.97 g P/day for infected calves. Although calves on both treatments showed positive P retention, the details of where this mineral is absorved and used was only possible with the quantification of P flows using radioisotopes. Although no significant differences were found in terms of endogenous loss and P absorption between healthy and infected calves, these seem to be the main mechanisms in the explanation for greater P in faeces and lower retention in infected calves.

P flow results between compartments are shown in Table 3. No significant differences were found between groups for these, due to the low number of calves used and higher standard error observed. Using this information, it was possible to quantify that the main destination of P was the central compartment (blood), represented by the readily available labeled form of the mineral, responsible for the maintenance of homeostasis in the animal organism, followed by bone tissue and finally soft tissues. Even in the control calves, P balance in the soft tissues and bone was negative, but guaranteed a growth rate of 190 g/day. This is low and was affected by quantity and quality of roughage offered. It should be noted that the proposed diet is in agreement with average diet intake for this type of animal on-farm in Brazil.

Although no significant differences were found in P flows between control and infected calves, the balance of P in bone tissue for the control group was negative (-0.53 g/day) and for the infected calves (-1.06 g/day). This showed, once again, the interference of the acute infection of *C. punctata* on P metabolism and placing bone tissue as the main mineral reserve used when this mineral is required by the animal. In a similar experiment, but with chronic infection of 10 000 L_3 per week during five weeks, the P metabolism was negatively affected the P kinetics, as a consequence of lower DM and P intake, as well as P retained. This led, more specifically, to weight loss, indicating a more severe process (Louvandini et al., 2009).

Studies in young sheep have shown that growth of bone tissue is guaranteed in detriment of adequate mineralization, leading to formation of bone with less maturity (Nicodemo et al., 1999), but maintained the animal development, as well as ensuring the vital functions for which P is essential. Therefore, the fact that calves gained weight is justified, despite the negative tissues balances. Bone and soft tissues are involved in this process, validating the hypothesis where mobilization of nutrients in young animals is directed towards growth. Nevertheless, this observation questions the P recommendation made by NRC (2001) for calves of about 100 kg body weight at 0.31% of DM intake, i.e. 8.3 $g.d^{-1}$ which was used to formulate the diet in this trial. Results here indicate that recommendations may not guarantee ideal deposition of P in the tissues (soft and bone). An acute infection by *C. punctata* negatively affected calf performance and P kinetics, increasing its excretion in the faeces and reducing its retention.

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Table 1. Chemical composition of diet received by calves

Chemical composition (g/kg dry matter)	Hay	Concentrate
Dry matter	915.63	923.72
Crude protein	75.82	153.15
Neutral detergent fibre	762.09	256.86
Ether extract Acid	403.75	93.11
detergent fibre	20.85	105.53
Ash	57.10	83.39
Phosphorus	1.78	10.81

Table 2. Intake, performance, plasma and P metabolism in control and calves infected with single dose of 45 000 L_3 of *C. punctata*

Variables	Treatments		se*	Probability
	Control (n=5)	Infected (n=5)		
Dry matter intake ($g.d^{-1}$)	2,297	2,297	0.06	0.71
P intake ($g.d^{-1}$)	12.43	12.43	0.0004	0.71
Live weight initial (kg)	66.30	65.80	1.58	0.80
Live weight final (kg)	70.30[a]	68.10[b]	0.61	0.03
Dairy gain ($g.d^{-1}$)	190.5[a]	109.5[b]	29.35	0.10
Plasma P (mg $.100^{-1}$mL)	7.80[a]	6.18[b]	0.50	0.05
Total protein ($g.100^{-1}$mL)	6.5	6.1	0.35	0.36
Albumin ($g.100^{-1}$mL)	3.5	3.6	0.35	0.78
Endogenous P in faeces ($g.d^{-1}$)	1.80	2.60	0.41	0.31
P absorbed ($g.d^{-1}$)	10.66	9.51	0.54	0.15
P excreted in faeces ($g.d^{-1}$)	3.75[b]	5.46[a]	0.65	0.08
P retained ($g.d^{-1}$)	8.68[a]	6.97[b]	0.64	0.09
Biological availability (%)	85.54	76.53	4.15	0.13

* standard error, [a and b] Means with different letters in the same row were significantly different ($P<0.10$)

Table 3. P flow in control and calves infected with a single dose of 45 000 L3 of *C. punctata*

Variables (P/g/day)	Treatments		se*	Probability
	Control (n=3)	Infected (n=3)		
P intake (F_{10})	12.43	12.43	0.0002	0.59
P faeces (F_{01})	3.41	4.83	2.33	0.30
P urine (F_{02})	0.00	0.00	-	-
P blood to GIT** (F_{12})	11.09	11.53	1.65	0.63
P GIT to blood (F_{21})	20.11	19.13	2.56	0.50
P blood to bone (F_{32})	3.64	2.68	1.27	0.56
P bone to blood (F_{23})	4.17	3.74	2.96	0.56
P bone balance	-0.56	-1.06	0.80	0.57
P blood to soft tissues (F_{42})	0.44	0.37	0.34	0.88
P soft tissues to blood (F_{24})	0.78	0.81	0.40	0.76
P soft tissues balance	-0.34	-0.44	0.14	0.78

* standard error; **gastrointestinal tract; [a and b] Means with different letters in the same row were significantly different ($P<0.10$)

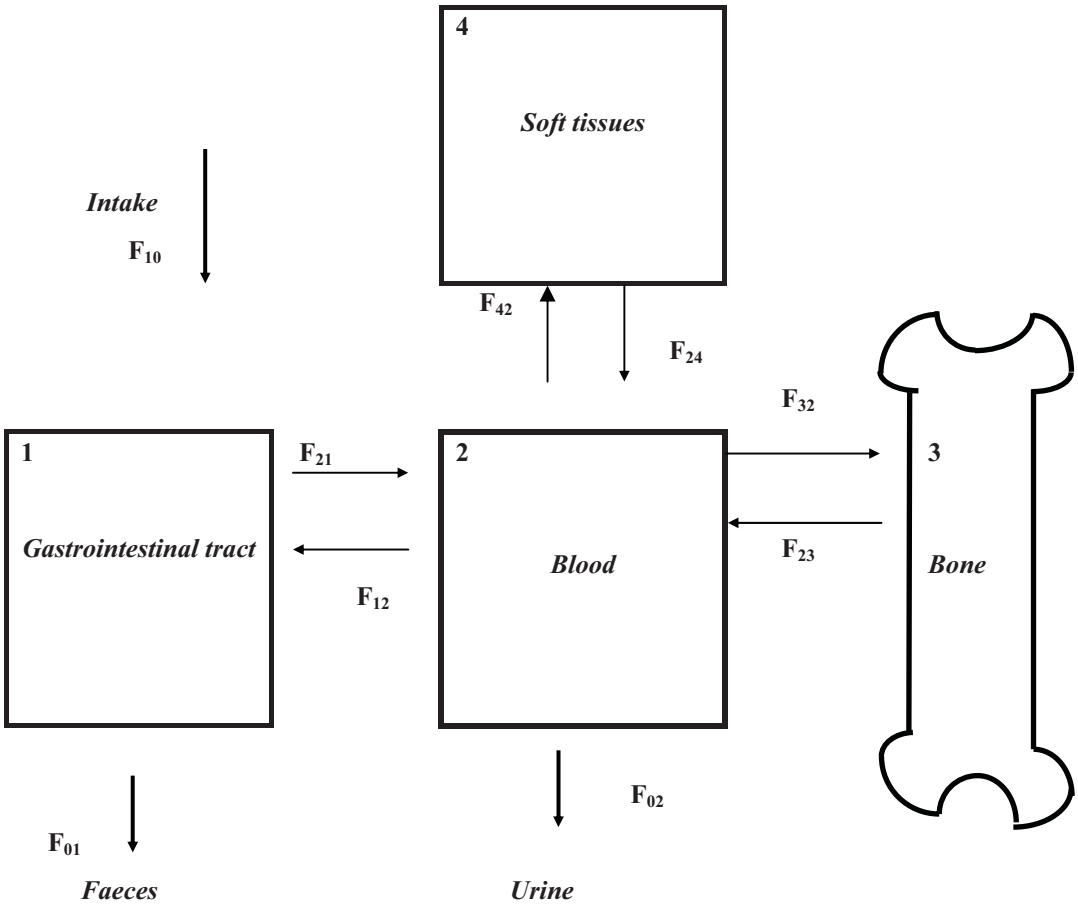

Figure 1. Schematic mathematical model for phosphorus (P) metabolism in calves, adapted from Vitti et al. (2000). Legend: P intake (F_{01}), P flow blood to gastrointestinal (F_{12}), P in faeces (F_{01}), P in urine (F_{02}), absorbed dietary P (F_{21}), P flow blood to bone (F_{32}), P flow bone to blood (F_{23}), P flow blood to soft tissues (F_{42}) and P flow soft tissues to blood (F_{24})

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The Effect of Different Densities of Planting on Morphological Characters, Yield, and Yield Components of Fennel (*Foeniculum Vulgare* Mill cv. Soroksary)

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Abstract

In order to study the effect of different densities of planting on yield, yield components and morphological characters of Fennel, an experiment was carried out in Karaj College of agriculture at 2008. Experiment was conducted based on completely randomized block design with three replications and five plant densities. Five plants spaces were 10, 15, 20, 25, and 30cm. Results indicated that the effect of plant density was not significant on the plant height, seed length and thousand seed weight. However, the effect of plant density on yield, number of umbel per plant and number of main branches was significant in 1% level. Maximum yield (2/431kg/plot), minimum number umbel per plant (67.26), and also minimum number of main branches (3/8) were obtained with maximum plant density. While minimum yield (1/315kg/plot), maximum umbel per plant (166.86) and maximum number of main branches (7/3) were obtained with minimum plant density.

Keywords: Fennel, Plant density, Yield, Seed weight, Umbel

1. Introduction

Fennel (*Foeniculum vulgare*) is a plant of the *Apiaceae* (*Umbelliferae*) family largely used to impart flavor to a number of foods, such as soup, sauces, pickles, breads, cakes, etc. Fennel is a shrub that measures 80- 150 cm of a height and has a pungent aroma. It is indigenous to the Mediterranean and is cultivated in England, Germany, Tyrol, China, Iran, Vietnam and South America. Diuretic, analgesic and antipyretic activity has also been found in the fennel fruit as well as antioxidant activity. The most frequently investigated was the essential oil which showed antioxidant, antimicrobial and hepatoprotective activity (Lucinewton et al., 2005). Present world market is around US$ 80 million. Iran exports these produce worth US$ 10 million (Masood et al., 2004).

Plant spacing is an important factor in determining the microenvironment in the fennel field. The optimization of this factor can lead to a higher yield in the crop by favorably affecting the absorption of nutrients and exposure of the plant to the light. .Gengaihi and Abdallah (1978) reported that number of umbel per plant, seed yield per plant and plant height was increased at the wider spacing. According to results of Verzalova et al (1988) row spacing did not effect on the plant height but number of umbel and seed yield per plant was increased at the wider spacing. Masood et al (2004) investigated the effect of row spacing (40, 50, 60, and 70cm) on morphological characters and seed yield of fennel and reported that the greatest plant height, seed yield per umbel, and seed yield per hectare were obtained with the lowest

row spacing but the lowest plant height, seed yield per umbel, and seed yield per hectare were obtained with the greatest row spacing.

Bianco and Damato (1994) reported that plant density not affected on plant height at flowering of primary umbel, number of stem and umbel per plant, yield per plant and per hectare. Hasanali et al (2002) with study the effect of different plant densities on yield dry material of Thyme (*Thymus vulgaris*) showed that the higher yield of dry material obtained with 15 cm densities of planting.

Aiello and Bezzi (1997) planted fennel at a spacing of 10, 15, 20, and 25 cm in rows and 75 cm apart and found no effect on plant establishment and survival in the first year but seed yield in the second and third years tended to be highest from plants 15 cm apart. Yadav et al (2000) conducted a field experiment on row and plant spacing and reported maximum plant height (182 cm), number of primary braches per plant (6.55), and number of umbels/plant (30.5) at 40x25 cm spacing.

Due to the medicinal and economic importance uses of fennel, the present trend is to increase the seed production and improve the quality of this crop. Keeping in view these facts, the present project was design to enhance the seed production of fennel under the agro climatic condition of Karaj (Table.1) by studying the effect of different densities of planting on morphological characters, yield and yield components on it.

2. Material and Methods

This experiment was carried out in the Tehran university, college of agriculture of Karaj in 2008 (Fig. 1). Field was plowed during the fall season and was disked before sowing time to provide a proper seedbed. Experiment was conducted based on completely randomized block design with three replications and five plant densities. The experiment includes 3 blocks and each block is contained 5 plots. Each plot size was 2.5x1.5m. Distance between blocks and plots were 1m. Five plant spaces were 10, 15, 20, 25, and 30cm. The distance among rows in all treatments was 40cm. Each plot was consisted of five rows. The bitter fennel seeds were sown at the 7th March 2008. Irrigation were done as: 1. 2-3 days interval irrigation until germination stage, 2. 4-5 days interval irrigation from germination to appearance first flowers stage, and 3. 7 days interval irrigation from appearance first flowers to harvest stage. Thinning was done when plants had 4-5 leaves. In order to better growing of plants, crust breaking operation were done at three stages (18th April, 4th May, and 19th May). All agronomic practices were keeping normal and uniform for all the treatments. Ten plants were selected at random from each plot for recording individual plant observation. Records in the growth stages were: plant height after appearance of first flower (Fig. 2-a) in many plants and after opening 50 percentages of flowers (Fig. 2-b), number of main branches, and number of umbel per plant. Then seeds harvested after ripening at two stages (20th August and 30th August) and dried in shade for 72 hour and measured the yield of seed per plot, length of seed, and weight of 1000 seeds. Data collected were analyzed using Duncan's test. Statistical software (SPSS) was used in order to analyze data.

3. Results and discussion

The effect of different densities of planting were significant on seed yield, number of main branches per plant, and number of umbel per plant at the 0.01 probability levels, but on Plant height, seed length, and thousand seed weight were not significant (Table.2).

3.1 Seed yield per plot

Different densities of planting had significant effect on the seed yield of fennel (Fig.3). Generally with decrease space among plants, Seed yield per plot increased. Maximum seed yield per plot (2.431 kg) was obtained with the highest plant density. While minimum Seed yield per plot (1.315 kg) was obtained with the lowest plant density. Although maximum seed yield per plot were obtained with the highest plant density but the highest seed yield per plant was obtained with the lowest plant density.

3.2 Length of seed

The length of seed was not affected significantly by different densities of planting. The maximum length of seed (5.1 mm) was recorded in 20cm among plant spacing. Minimum length of seed (4.06mm) was recorded in 30cm among plant spacing (Fig.4).

3.3 Weight of 1000 seeds

Effect of different densities of planting on 1000-seed weight was not significant. The maximum weight of 1000 seeds (8.26 gr) was recorded in 20cm among plant spacing. Minimum weight of 1000 seeds (6.03 gr) was recorded in 30cm among plant spacing (Fig.5).

3.4 Number of umbel per plant

Number of umbel per plant was affected significantly by different densities of planting. Generally with increase space among plants, number of umbel per plant increased. The maximum number of umbel per plant (166.86) was recorded in lowest plant density. While minimum number of umbel per plant (67.26) was recorded in highest plant density (Fig.6).

3.5 Number of main branches per plant

Our measurements showed that different densities of planting had also an expressive effect on the number of main branches per plant. Generally with increase space among plants, number of main branches per plant increased. The maximum number of main branches per plant (7.3) was recorded in lowest plant density. While minimum number of main branches per plant (3.8) was recorded in highest plant density (Fig.7).

3.6 Plant height after appearance of first flower in many plants and after opening 50 percentage flowers

Plant height in different growth stages was not affected significantly by different densities of planting. But the maximum plant height in different growth stages was recorded with maximum plant density. While the minimum plant height was recorded with minimum plant density. Maximum plant height after appearance of first flower (28.86 cm) and after opening 50 percentage flowers (89.7 cm) was belonged to 10 cm plant spacing. Minimum plant height after appearance of first flower (19.96 cm) and after opening 50 percentage flowers (74.53 cm) was belonged to 30 cm plant spacing (Fig.8 and Fig. 9).

4. Conclusion

Fennel plant is one of the most interesting research plants. It is between medicinal and aromatic plant. Plant density is an important factor in determining yield of fennel. Results of this experiment showed that influence of different densities of planting on seed yield, number of main branches per plant, and number of umbel per plant were significant. The lowest plant density produced higher number of main branches, seed yield, and umbel per plant. While the higher seed yield per plot was belonged to the highest plant density. These results are supported by the findings of Gengaihi and Abdallah (1978), Verzalova et al (1988), and Masood et al (2004). But these results are in contrast by the findings of Bianco and Damato (1994), and Akbariani et al (2006). Plant height, seed length, and thousand seed weight not affected significantly by different densities of planting. These results are in agreement with findings Bianco and Damato (1994). Akbarinia et al (2006) with study the effect of plant density on seed yield, essential oil and oil content of Coriander (*Coriandrum sativum* L.) showed that with increasing of plant density, seed yield and oil content had a significant decrease. Heidari et al (2008) reported that in peppermint (*Mentha piperita* L.) dry yield increased by increasing the plant density. Baldwin and Wesley (2006) suggested that in Kenaf (*Hibiscus cannabinus* L.), the narrowest row spacing of 35.5 cm, gave the greatest biomass yield as well as the highest bark yield per hectare. Lebaschy et al (2008) with study the effect of plant density on growth indices of Safflower (*Carthamus tinctorius* L.) showed that the maximum dry matter was obtained in the highest density but the maximum RGR (relative growth rate) and CGR (crop growth rate) were observed in low density. Gimplinger et al (2008) reported that in Amaranth (*Amaranthus cruentus*), total shoot biomass did not respond to crop density but the highest grain yield was obtained at the lowest plant population. Generally in order to gain maximum fennel yield, plants must be cultivated with 10 cm space between them. But more research is needed for understanding the effect of plant density on oil and essential oil content of fennel.

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Table 1. Geographical coordinates, average annual rainfall and mean annual temperature of Karaj

longitude	latitude	height of sea level(m)	average annual rainfall(mm)	mean annual temperature(°c)
50° 59′E	35° 47′N	1312/5	230	14/3

Table 2. Analysis of variance for morphological characters, yield, and yield components of fennel

Source	d.f	Yield of seed per plot	Length of seed	Weight of 1000 seeds	Number of main branches	Plant height after appearance of first flower	Plant height after opening 50 percentage flowers	Number of umbel per plant
Repeat	2	58503/8	0/416	0/715	0/641	99/22	29/64	284/32
Treat	4	614414/9**	0/544ns	2/538^{ns}	5/302**	40/54^{ns}	102/28^{ns}	4539/5**
Error	8	75142/2	0/299	1/536	0/566	27/14	26/91	522/3
C.V.	-	16	11/34	16/17	13/05	21/81	6/30	18/34

**: significant responses at the 0.01 probability levels ns: not significant

Table 3. The effect of different densities of planting on morphological characters, yield, and yield components of fennel

Factor / Space between plants	Yield of seed per plot	Length of seed	Weight of 1000 seeds	Number of main branches	Plant height after appearance of first flower	Plant height after opening 50 percentage flowers	Number of umbel per plant
10	2431/06[a]	5/03[a]	8[a]	3/8[a]	28/86[a]	89/7[a]	67/26[a]
15	1841/3[b]	4/93[a]	8/03[a]	5/5[b]	26/53[a]	85[ab]	105/86[ab]
20	1611/7[b]	5/1[a]	8/26[a]	5/6[b]	21/93[a]	83/8[abc]	136/23[bc]
25	1368/5[b]	4/96[a]	8[a]	6/63[bc]	22/13[a]	78/86[bc]	146/8[bc]
30	1315/2[b]	4/06[a]	6/03[a]	7/3[c]	19/96[a]	74/53[c]	166/86[c]

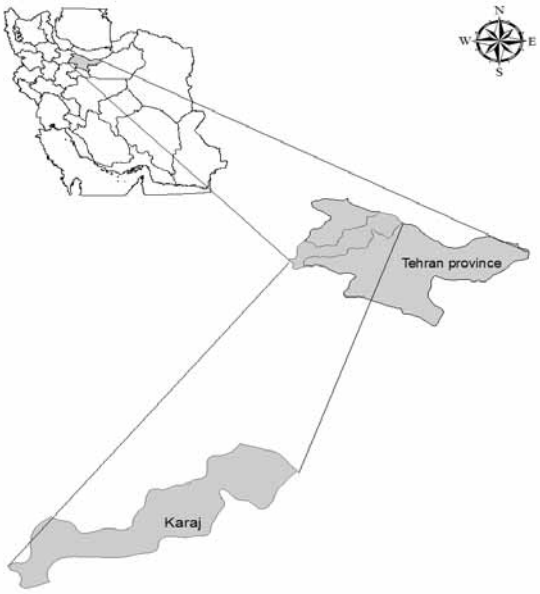

Figure 1. The location of Karaj

Figure 2. a. Appearance of first flowers b. Opening of 50 percentages of flowers

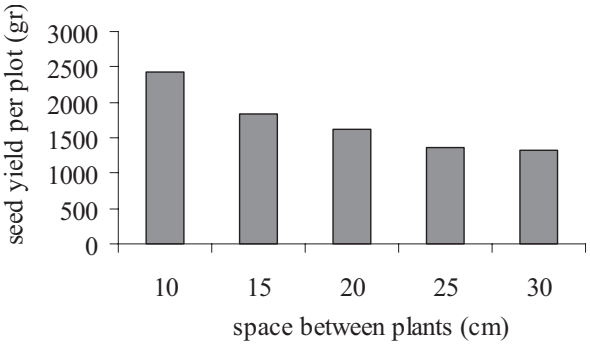

Figure 3. Effect of space between plants on Seed yield

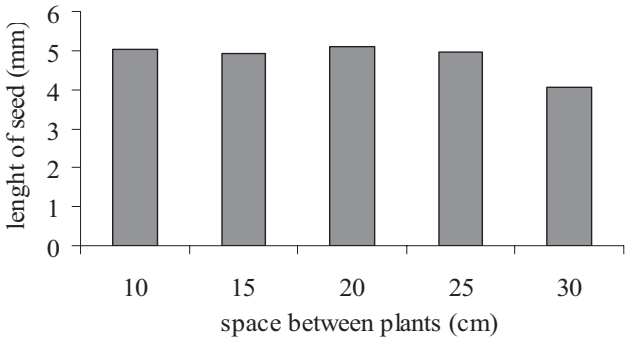

Figure 4. Effect of space between plants on length of seed

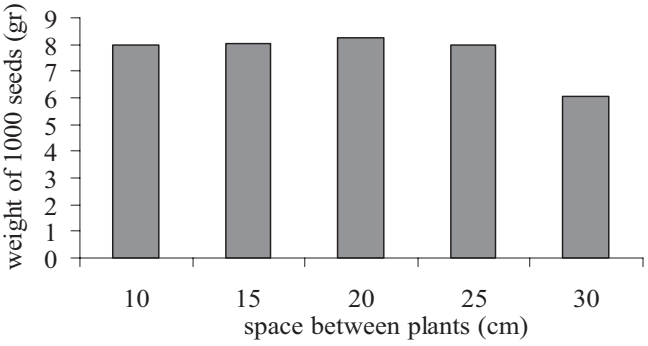

Figure 5. Effect of space between plants on weight of 1000 seeds

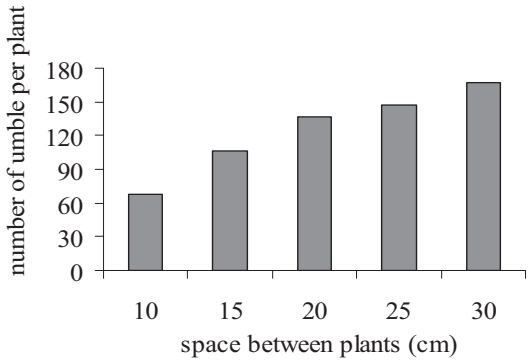

Figure 6. Effect of space between plants on number of umbel per plant

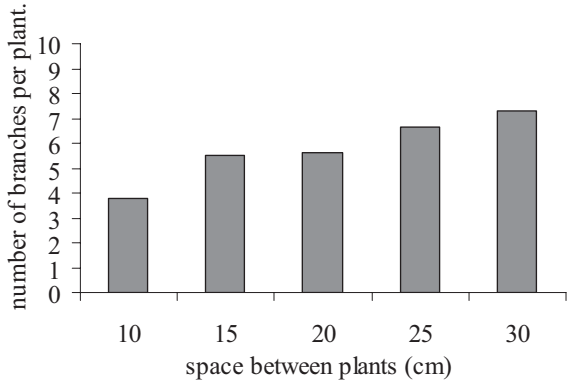

Figure 7. Effect of space between plants on number of main branches per plant

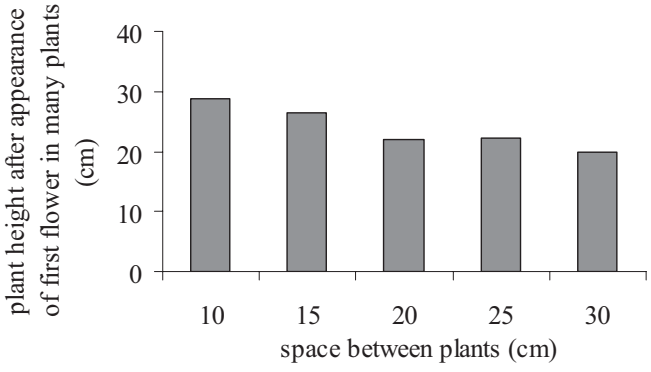

Figure 8. Effect of space between plants on plant height after appearance of first flower

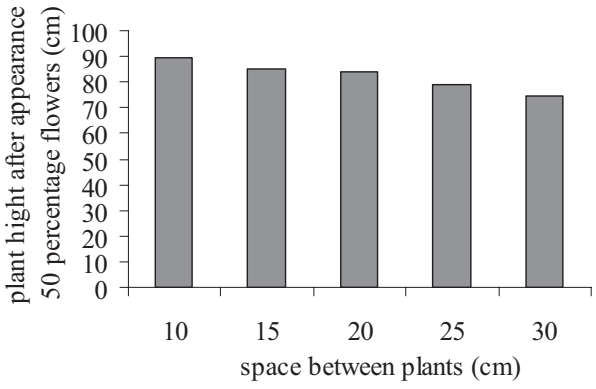

Figure 9. Effect of space between plants on plant height after opening 50 percentage flowers

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Aboveground Biomass of Selected Provenances of *Acacia Mangium* and *Acacia Aulacocarpa* Multiple-leadered Trees

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Abstract

Acacia mangium Willd. and *Acacia aulacocarpa* A. Cunn Ex. Benth are two important Acacias for biomass production. Being multiple-leader (ML) and fast growing species, both species are the best bet for carbon sequestration and bio-energy supplementary. The main objective of this study was to assess the aboveground biomass and to derive aboveground biomass equations of these species and provenances. Destructive sampling was carried out with 36 samples per species and diameter at breast height (Dbh) and categorized into three classes namely small (11-15 cm), medium (16-20 cm) and big (21-25 cm) for *A. mangium* and 6-10 cm, 11-15 cm and 16-20 cm for *A. aulacocarpa* respectively. *A. mangium* from SW of Boset WP (PNG) produced 380.83 t/ha of aboveground biomass, Captain Billy Road (QLD), Bansbach WP (PNG) (224.44 t/ha) and Russel and Gap CK (QLD) (49.63 t/ha) while for *A. aulacocarpa*; provenance from Arufi E Morehead WP (PNG) (171.88 t/ha), W. Morehead (PNG) (150.90 t/ha), Samford (QLD) (63.87 t/ha) and 3K S Mt. Larcom (QLD) (25.32 t/ha) respectively.

Keywords: *Acacia mangium*, *Acacia aulacocarpa*, Multiple-leader, Aboveground Biomass, Provenance

1. Introduction

Estimating tree and forest biomass is essential for assessing ecosystem yield and carbon stock in compliance with the Kyoto Protocol on greenhouse gas reduction (Korner, 2005). Biomass is being frequently used to quantify traditional forest products (Guttenberg, 1973; Husch *et al*., 1982) and as estimated value of wood as a raw material. Therefore determining biomass is a useful way of providing estimates of the quantity of these components. Biomass studies for different forest types in the world have been intensified under the International Biological Programme (IBP) in the 1970s (Brown, 1997).

Currently, the forest biomass studies have increased in the past several years as basic ecosystem data that are needed for the development of sound ecological land management and to predict the dynamics and productivity of the forests (Melillo *et al*., 1993). Prior to 1980, biomass information in the world was rather limited in the tropical countries. In recent years, biomass studies in the tropics have been conducted by many researchers such as Kato *et al*. (1978), Kawahara *et al*. (1981), Brown *et al*. (1991), and Brown (1997). They found that the biomass production varied among species and sites. The species-site interactions were the main concern to use the established biomass equations.

Ledin and Willebrand (1996) noted that maximum biomass production can be achieved by optimizing genotype and/or cultural management and it can be viewed over a spectrum of spatial or temporal scales (Harris *et al*., 1973). At the largest scale, entire region are being examined to determine (i) the rate of succession, (ii) productivity profiles, (iii) the potential impacts of various harvest strategies on production and biomass (Sharpe and Johnson, 1973), (iv) carbon cycle (Brown, 1997) and energy supply (Jackson and Jackson, 1997). While at the smallest scale, the physiological processes such as photosynthesis, respiration and decomposition which are integral to biomass have been studied. These could help to establish their *in situ* relationship to environmental variables, biomass accumulation and turnover (Harris *et al*., 1973).

In this study, we examined the accumulation of aboveground biomass and also developed equations based on growth data in four provenances of *Acacia mangium* and *A. aulococarpa* from two regions in a progeny trial. *Acacia* species have shown tremendous growth performance in many research sites and provenance trials (Thinh *et al*., 1998; Bino, 1998). These species grow well on both lowland and highland especially in Vietnam and Papua New Guinea. *A. mangium* produces height of 25 m to 35 m with straight bole and the diameter at breast height (Dbh) can be over 60 cm. However, on relatively poor sites the trees are usually much smaller, with average heights between 7 m to 10 m (Turnbull, 1986). On the other hand, provenance trials of *A. aulacocarpa* conducted at Cebu Province, Philippines revealed that the species could reach 6.5 m after two years of planting (Baggayan and Baggayan, 1998), but exhibited poor survival of only 38%. After 5 years in the field trial at Da Chong, Dong Ha and La Nga at Vietnam, *A. mangium* outperformed *A. aulacocarpa* where the former species recorded 10 cm in means of respectively Dbh and 8.1 m in height while the latter species recorded 7.2 cm and 6.0 m respectively (Nghia and Kha, 1998).

2. Materials and Methods

2.1 Site Selection

The study area utilized a progeny trial which was established on August 1998 in How Swee Estate, Kampung Aur Gading, Kuala Lipis, Pahang Darul Makmur which is located about 130 km from Kuala Lumpur. The latitude and longitude of the trial are 4° and 20.5' N and 101° and 55.5' E. Previously, the estate was planted with rubber trees (*Hevea brasiliensis*). The estate area is situated approximately 91 m above sea level. In general it has a uniform topography and considered as a flat area. It receives mean annual temperature, relative humidity and rainfall of 30°C, 70% and 2515.28 mm respectively.

2.2 Plant Materials

This study utilized plant materials of a seventh year old progeny trial involving two important *Acacia* species namely *Acacia mangium* and *Acacia aulacocarpa*. This study only focused on two out of four species planted due to their high occurrence of ML formation. For each species, there are four provenances, thus a total of eight provenances for this study. To overcome the confounding effect, every samples were classified into Dbh classification. This is due to the establishment of the research area which was based on provenance/ genotype trial which every species/ provenance/ genotype were assigned randomly within lines and blocks. The samples located at the verge of the block were removed from the selected samples. The original sources of these seeds were supplied by the Commonwealth Scientific and Industrial Research Organization (CSIRO).

2.3 Assessment of the aboveground biomass (AGB) of Acacia mangium and Acacia aulacocarpa multiple- leadered trees

Aboveground biomass evaluation was based on mean biomass per provenances, AGB equations for each provenance and the AGB estimation based on one hectare with the tree spacing of 3 m x 3 m apart. Details of assessment of this parameter are given as follows:

Prior to determination of performance, total enumeration on their Dbh and height was carried out to assess the overall variation and distribution of ML trees within the species and provenances. The data were sorted accordingly from the lowest to the highest range Dbh and height. Then, they were divided into three classes according to Dbh groupings to hinder the confounding effects. For each group, the mean value of Dbh and height were calculated. Three samples with the nearest values to the average value of each class were chosen as samples to represent each group. Then, this was followed with ground checking to verify and identify these samples. The selected trees were then marked with ribbon for the purpose of identification in the field.

The diameter at breast height was classified into three classes namely small, medium and big. They are: Small (11-15 cm), Medium (16-20 cm) and Big (21-25 cm) for *A. mangium* and 6-10 cm, 11-15 cm and 16-20 cm for *A. aulacocarpa* respectively. In each class; there were three replications, thus making a total of 9 ML trees per provenance utilized in this experiment. There were 72 ML trees involved in this destructive sampling. ML class 2 was utilized in this experiment as it was only under this category which contained sufficient numbers of trees to be assessed for this study.

Before the trees were felled, the area above ground level was cleared and cleaned to avoid any sample contamination. A chain saw was used to fell the trees and cut the components to a smaller size. Then, samples of biomass were divided into three components namely stem, branch/ twigs and foliage. All the components were weighed to obtain the total fresh weight. The main stem was further divided into three parts namely bottom, middle and top. A 10 cm disc wood from each part was weighed for dry weight. 100 g of foliage were taken in three replicates using Digi Digital Weighing Scale (1.5 kg ± 0.05 g). Samples of wood disc and the foliage were oven-dried at 108^{o}C for 48 hours (memmert, 200^{o}C ±0^{o}C) until constant weight was achieved (Kato *et al*. 1978). The samples were then subjected to the follow equation to determine their biomass production:

$$\text{Biomass} = [\text{DW/FW} * 100\%] * \text{TFWC}$$

Where:

FW : Fresh Weight (kg)

DW : Dried Weight (kg)

TFWC : Total Fresh Weight Components (kg)

Then the components of biomass were summed up to obtain the estimation of aboveground biomass (AGB). Diameter at breast height and height were incorporated to derive AGB equations using Multiple Linear Regression (SPSS ver. 12). The multiple linear regressions were used to predict for the variance in the interval dependent, based on linear combinations of interval, dichotomous or dummy independent variables. Biomass estimates employed in this study followed a Dbh-based regression established by Kato *et al*. (1978). Most biomass studies using allometric relations have utilized Dbh as the independent variable because of its ease of measurement and direct relation to tree growth (Jumanne *et al*., 1983).

3. Results and discussions

The results for both species were analyzed separately because they were considered as different entities in relation to different responses to treatment.

3.1 Aboveground biomass for Acacia mangium

From the analysis of variance (ANOVA) of aboveground biomass (AGB) components of *A. mangium* ML provenances indicated significantly differences at $P \leq 0.05$ between provenances for stem and total biomass and on the other hand, for Dbh classes; all the parameters measured were significantly different (Table 1). However, the interaction between

provenance and Dbh classes were insignificant for provenance differences. This could infer that provenances and Dbh classes were not inter-related or dependent on each other. In addition, Figure 1 shows the summary of mean values of AGB component of *A. mangium* ML provenances and Dbh classes based on per standing trees. Provenance from SW of Boset WP, PNG produced higher mean of AGB (400.19 kg) followed by Bensach WP, PNG (234.15 kg), Captain Billy Road, QLD (211.95 kg) and Russel & Gap CK, QLD (202.68 kg). The DMRT also shows that the amount of total AGB and stem biomass for SW of Boset WP (PNG) was significantly different from other provenances. However there was no significant difference between provenances with regards to other variables such as branch/ twigs and foliage biomass. The Dbh classification also shows that the total biomass and stem biomass were accordingly to Dbh classification namely big, medium and small Dbh classes. On the other hand, there was no significant different for Dbh classes of big and medium with regard to branch/ twigs and foliage biomass.

3.2 Aboveground biomass for Acacia aulacocarpa

Similarly for *A. aulacocarpa* ML provenances also showed significant differences at $P \leq 0.05$ between provenances, Dbh classes and interaction between them (Table 1). However, the branch and twigs were not significant with regards to their interactions factors. Figure 2 shows the summary of mean values of AGB components of *A. aulacocarpa* ML provenances and Dbh classes. Provenance from Arufi E. Morehaed WP gave the highest total of aboveground biomass (219.72 kg) followed by W. Morehead, PNG (209.03 kg), Samford, QLD (91.25 kg) and 3K S Mt Larcom, QLD (34.12 kg). Provenances from Papua New Guinea shows were significant different from the ones from Queensland in terms of stem, foliage and total AGB. The Dbh classes showed that the Dbh "big" class was significantly different from the other two classes of Dbh for stem biomass, foliage biomass and total AGB. In addition, there is no significant different for classes of Dbh "medium" and "small" with regards to branch and twigs biomass.

3.3 Correlations and biomass equations

A Pearson's Correlation Coefficient was conducted with regards to AGB and growth parameters. It was found that Dbh and tree height was highly correlated with AGB for both species ($R^2 > 0.96$). In addition, eight regression equations were also projected to estimate AGB per one hectare basis and they were shown in Table 2. Again, *A. mangium* from SW of Boset WP (PNG) was found to outperformed the other provenances and recorded the highest estimated AGB per hectare of 380.83 tons/ ha followed by Captain Billy Road (QLD) 251.17 tons/ ha, Bensbach WP (PNG) 244.44 tons/ ha, and Russel and Gap CK (QLD) 49.63 tons/ ha. SW of Boset WP (PNG) provenance was more than 700% better compared to Russel and Gap CK (QLD) provenance in terms of total AGB. The occurrence could be due to the influence of the stress trees where slow performed trees such as the ones from Russel and Gap CK were actually suppressed by the good performing trees from other provenances. Meanwhile for *A. aulacocarpa*, provenance from Arufi E. Morehead WP (PNG) produce the estimated of biomass of 171.88 tons/ ha and followed by W. Morehead (PNG) 150.90 tons/ ha, Samford (QLD) 63.87 tons/ ha and 3K S Mt Larcom (QLD) 25.32 tons/ ha respectively.

In addition, the regression analysis for both species revealed that there are strong correlations between total aboveground biomass and Dbh in all provenances ranging from 0.81 to 0.98 for *A. mangium* and from 0.74 to 0.99 for *A. aulacocarpa*. The multi-linear regression was also constructed using the interactions of Dbh and tree height but the correlations results were found to be relatively low if only Dbh was used. The statement was supported by the correlation results where it is proven that Dbh was strongly correlated with AGB compared to height (Table 2). The AGB equations clearly showed that seven out of eight provenances of this study showed that Dbh was the main factor supporting the equations which was actually similar to Zianis and Mencuccini (2002) on *Fagus sylvatica*. Interestingly, there was higher amount of AGB being showed lower by R^2 compared with the lower amount of AGB in *A. mangium*. Both species showed strong equations correlation based on Guilford's Rule of Thumb (Guilford, 1956). Even though the results of the estimated AGB could be used as early indicator for the species/provenances performance but site-dependent variation in biomass accumulation have been reported by Kondo and Oshima (1981) for *Helianthus tuberosus* species and three Betulaceae species by Walters *et al*. (1993). They concluded that, there were significantly different in terms of biomass productions with regards to species and sites.

4. Conclusion

Based on the results, aboveground biomass of *Acacia mangium* and *Acacia aulacocarpa* were subjected to diameter at breast height (Dbh) rather than tree height. Even though the aboveground biomass production of *A. mangium* relatively higher than *A. aulacocarpa*, further study should be undertaken to assess the calorific value of these species. This is because, the absolute performance in terms of energy is not the amount of wood in kilograms or tonnes per hectares but depends on the calorific value per gram of wood burned.

5. Acknowledgement

We have to remember Mr. Salim, Mr. Zakaria and Mohd Noor Hisham for their help during the field experiment and graphic design. This project wouldn't be reality without supports from CSIRO for providing plant materials. The project was funded by MOSTI under the competitive grant IRPA/EA 0009913.

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Table 1. Analysis of variance (ANOVA) of AGB components of *A. mangium* and *A. aulacocarpa* ML provenances and Dbh classes

Source of variations	DF	Stem Biomass (kg)		Branch and Twigs Biomass (kg)		Foliage Biomass (kg)		Total Biomass (kg)	
		Mean square	F Value	Mean square	F Value	Mean square	F Value	Mean square	F Value
A. mangium									
Provenance	3	46483.64	7.19*	93.08	0.25ns	70.98	1.22ns	51789.14	5.62*
Dbh Class	2	182012.93	28.16*	1765.79	4.66*	366.15	6.29*	232998.37	25.30*
Provenance*Dbh	6	13099.33	2.03ns	152.53	0.40ns	24.60	0.42ns	13972.87	1.52ns
A. aulacocarpa									
Provenance	3	28821.07	17.33*	1163.53	3.88*	235.93	17.59*	47670.53	17.52*
Dbh Class	2	43365.65	26.08*	2971.36	9.91*	269.05	20.06*	77880.59	28.62*
Provenance*Dbh	6	9907.08	5.96*	343.38	1.15ns	63.64	4.75*	14753.61	5.42*

*Significantly different at P≤0.05

Table 2. The summary of aboveground biomass equations

Species	Provenance	Equation	R^2	Estimated Biomass/ ha
A. mangium	Bansbach WP (PNG)	y = 15.1x – 161.71	0.8085	224.44
	SW of Boset WP (PNG)	y = 24.538x – 314.52	0.909	380.83
	Captain Bily Road (QLD)	y = 14.743x – 130.5	0.9256	251.17
	Russel and Gap CK (QLD)	y = 21.966x – 254.02	0.976	49.63
A. aulacocarpa	W. Morehead (PNG)	y = 18.504x – 182.32	0.9923	150.90
	Arufi E Morehead WP (PNG)	y = 21.175x – 213.59	0.9864	171.88
	3K S Mt Larcom (QLD)	y = 6.5299x – 29.432	0.955	25.32
	Samford (QLD)	y = 11.436x – 55.678	0.7427	63.87

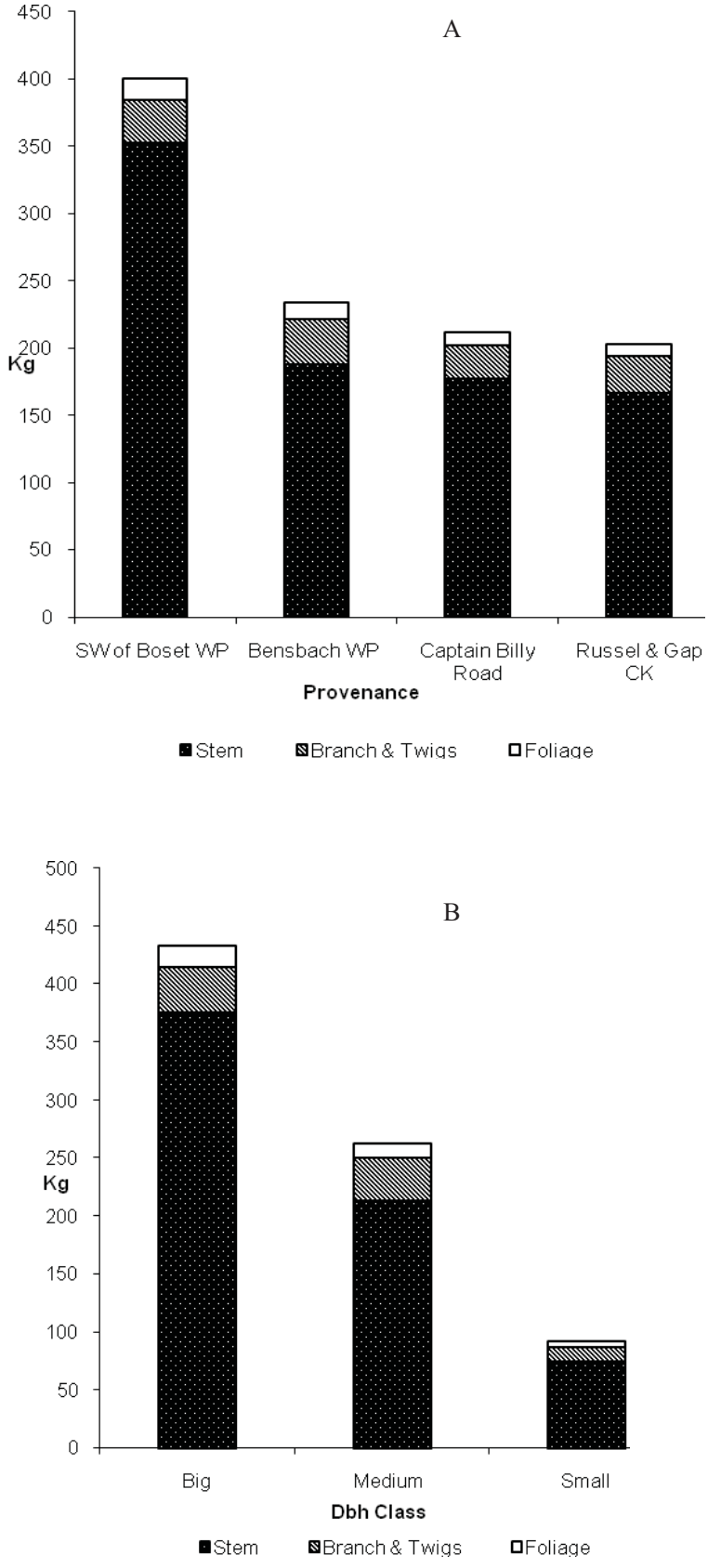

Figure 1. Total aboveground biomass of *Acacia mangium* with regards to provenances (A) and Dbh classes (B)

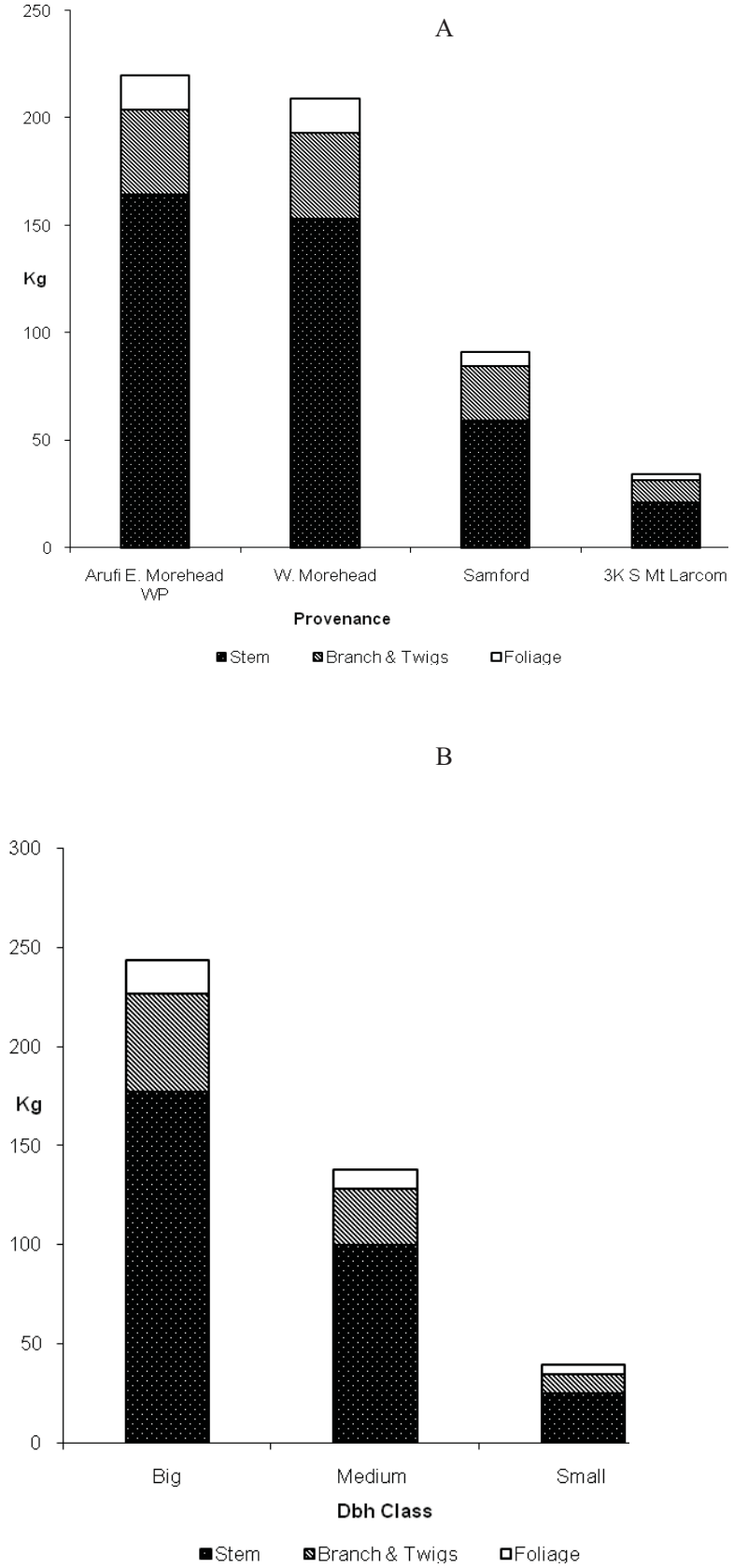

Figure 2. Total aboveground biomass of *Acacia aulacocarpa* with regards to provenances (A) and Dbh classes (B)

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Statistic Characteristics of Chinese Provinces' and Municipalities' Agriculture Sector at 2008

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Abstract

This paper selected several agricultural development indicates in China at 2008 to do statistical analysis. Demonstrated by the new type of data table graph, some information is showed, and the distributed rule of several indicates are described. Agricultural development regions are classified according to Q-type Cluster. The corresponding analysis methods are applied to gain the relationship among the provinces and municipalities and variables. Moreover, some revelations are educed.

Keywords: Statistical analysis, Distributed rule, Chinese agriculture

1. Introduction

In 2008, under the guidance of Deng Xiaoping Theory, thoroughly implement the scientific concept of development, farmers in the provinces and municipalities strive to overcome the serious natural disasters that rarely seen in history and the impact of the international financial crisis, making economic development of agriculture keep fast. The future development's most difficult task of building a well-off society in an all-round way in our country is still the "three rural" problem. It is of great significance to sum up the rule of the distributed rules of agriculture at every province and municipality, for sustained, rapid, healthy and coordinated agricultural development in the future.

2. The DING Chart (Ding, Yuechao, 2007) of original data

According to "China Statistical Yearbook 2008", the original data include Cereal, Beams, Tubers, Oil-bearing Crops (OBC), Fruit, Cattle, Pig, Sleep, Total Aquatic Products (TAP), and Animal Husbandry (AH). The original data has 10 indicators, 31 records. It is difficult to analyze in horizontal and vertical and to find the rule of the distribution. For too much indicators and data, the ordinary histogram, broken line maps, graphs, pie-chart, have been power less. Now use the newly created grid-form chart to display information (Figure 1). The figures in each grid of the intuitionist chart are transferred into ovals to indicate the relative size of the data, through standardization of the data to ring from 0 to 1. Each field indicators for the smallest is only a line, the largest grid for a full oval.

It can be seen directly from the Figure 1, the ovals of Heber, Shandong, Henan and Sichuan are bigger than others. These provinces are the major agricultural province in China. Agriculture in region of Beijing, Tianjin, Shanghai, Tibet, Qinghai, and Ningxia is backward. On the whole, agriculture in the northeast, north China, east China, central China (except municipalities of Beijing, Tianjin and Shanghai) is prosper compared to the South China, Southwest, Northwest region.

It also can be clearly seen from Figure 1 that the production of grain in the northeast, north China, east China and central China is higher, while that in the north China, southwest and northwest region is lower. Heilongjiang is the main area of Beams, the production here is the highest of China. The production of Tubers in Sichuan and Chongqing is higher than any other place, because it is suitable for drought-resistant and barren-resistant Beams to grow in the basin, hilly and dry land of Sichuan. Oil-bearing Crop and fruits in Shandong and Henan be produced most, while the number of cattle in the municipalities and East China is only a little and only a few pigs in the southwest at the end of year 2008. Inner Mongolia and Xinjiang where grasslands are more common have more sheep than other place. Total Aquatic products are proper in the coastal areas.

3. The divisions of similar regions

Through Q-type Cluster Analysis, The analysis of the similarity of the development of agriculture characteristics among the regions can be got. The 31 provinces' and municipalities' pedigree map is showed in figure 2, the similarity of the indicators is measured by similar coefficient. The similar characteristics samples will be clustered together by the

Q-type Cluster Analysis. Most pasts of East China, Central China, South China are clustered to class I, while northeast, North China, southwest and northwest are clustered to class II, only a few specific regions have some fluctuation. Seen from Ding Chart, the size of oval of class I is big and same, while that of class II is various and small.

Combination of the division and analyzing the DING Chart, some rules and characteristics are easier to be found out.

The characteristics of class I: all the agricultural production development balanced, and the production of most part of the regions is higher. This is because South China, Central China and East China own large area of plain and vast river which have plenty of water for irrigation. In southern coastal area, the weather is hot and rainy, which has a long frost-free period that crops can grow well throughout the year. In all, the climate and the geographical location of these areas are helpful, making agriculture, forestry, animal husbandry and fishery be able to be better and more balanced development.

Agricultural production in these areas is higher than that of other areas. These areas' agriculture developed well, which is China's major agricultural production areas.

Northeast, North China, southwest, northwest cluster into class II, agricultural production in these areas is Unbalanced. From Figure 1, the sizes of the ovals of these areas are different and non-uniform and the size of the ovals are small, indicating these areas are low-yield. Northeast, where is the base commodity grain production location, has a long period of cold winter and a warmth summer that the sunshine is long. The climate in North China is dry; as a result, most of the areas are shortage of water for irrigation. Most part of the southwest are subtropical, and southern Yunnan is tropical and vertical difference is marked that make a mountain climate. The agricultural in Qinghai-Tibet area restricted by the climate, which makes it unsuitable for crop, however, the animal husbandry develop better compared with others. The grasslands are mainly distributed around the lakes and rivers.

The special climate and geographical position make agriculture in these areas varied and backward, especially for marine production.

4. The explanation of the relationship of variables and regions

Correspondence Analysis can reveal the differences between the various categories of the same variables and the correspondence relationships between different variables and various categories. In order to facilitate the observation and analysis, we make the factors of the R-load and Q-load matrix into the more intuitive scatter plot (Figure 3). The scatter plot can be divided into three sections, thus some conclusions can be got.

Guangdong, Fujian, Hainan, Shandong and Zhejiang: marine production and fruit develop well. Marine production and fruit requite water and good climate. Coastal climate and the vast ocean provide proper condition for marine production and fruits. The coast of East Sea and Yellow Sea is a good place for fishing, where owns various species of fish, and the ground is flat and the depth is shallow. Fishers here find out the marine fish's "temper", using "one divides into two" to analysis the relationship between the wind and the fish, insisting "publicity stunts, catch the wind tail" in order to get marine fisheries harvest.

Shanxi, Ningxia, Gansu, Qinghai, Tibet, Inner Mongolia and Xinjiang: sleep and cattle indicators are high, indicating a better development of animal husbandry in these areas. Sheep and cattle are herbivores, and these areas are in western China, where the grasslands, grass-mountain possess the main part of country's total area. The western regions have the advantages over the animal husbandry resources, which provide the western a broad prospect. And the western make full use of the large area of grass to develop the animal husbandry and achieve the coordination of configuration for agricultural resources between the east and the west to form the agricultural division and cooperation in the regional economy.

Other provinces and municipalities: a combination of grain, beans, potato, oil and pigs, animal husbandry. Pig and animal husbandry provide organic fertilizer for these crops, making soil more fertile. And the soil horizon in these areas is deep, higher fertility of black soil is all over the areas that is beneficial to the growth of agricultural mechanization, thus the crops grow better.

5. Conclusion

From the 2008 Chinese provinces' and municipalities' agricultural development's DING Chart, some conclusions can be made. Heber, Henan, Shandong, Sichuan is the major agricultural province, while the western provinces' and municipalities' agricultural are backward. From the vertical perspective, the national agricultural development can be divided into two regions. The indicators of region I develop balanced and well, while that of region II is various and backward. Though correspondence analysis, we can conclude that agricultural development is different, every region has its own characteristics. Making full advantage of the characteristics of every province and municipality to develop the specialty agriculture make our country's agriculture grow sustained, rapid, healthy, coordinated.

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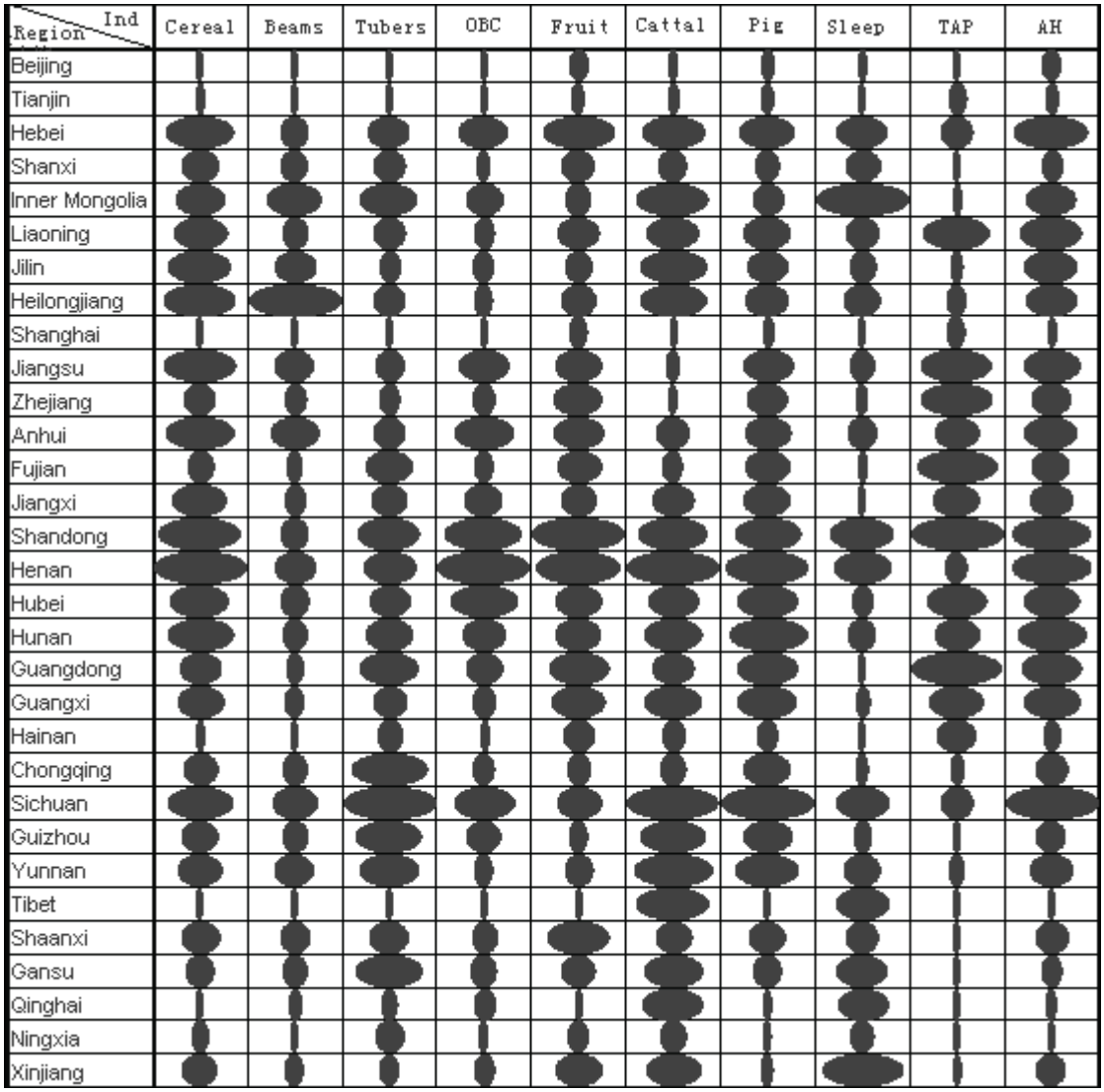

Figure 1. DING Chart of Chinese provinces' and municipalities' agricultural indicators at 2008

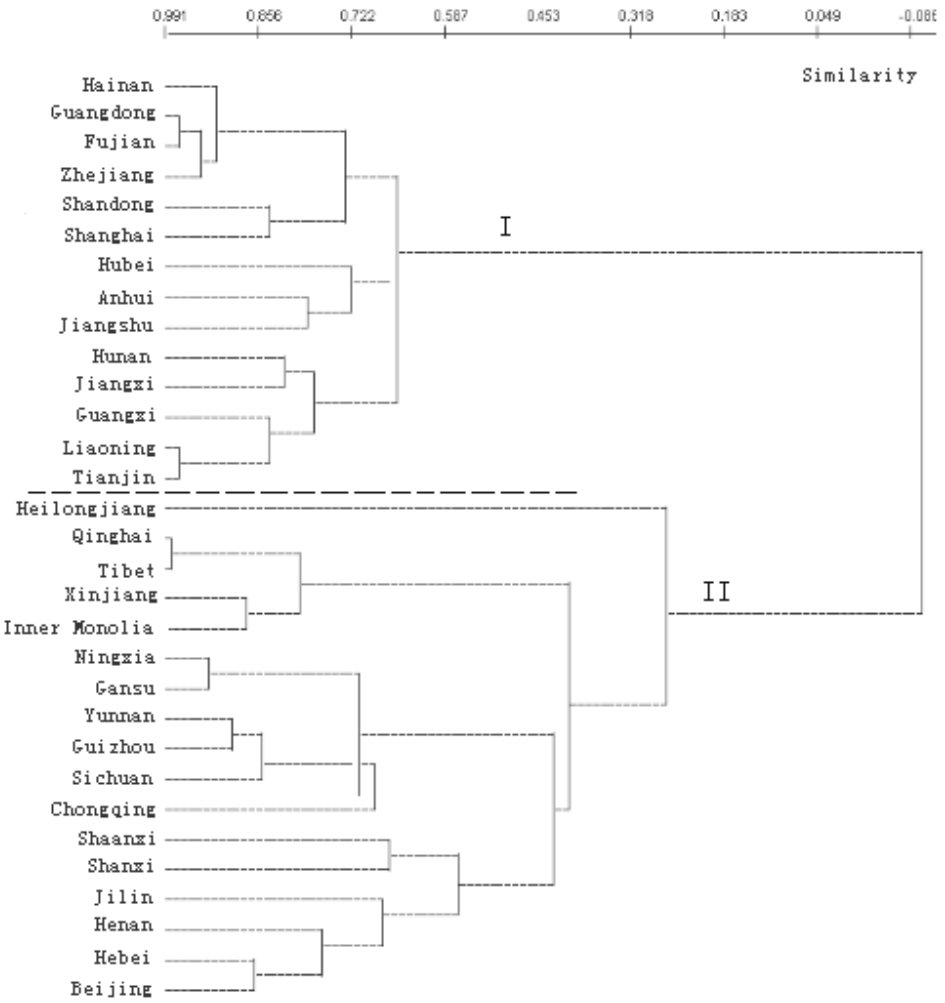

Figure 2. The Q-cluster pedigree map of the similarity of agricultural characteristics in every region

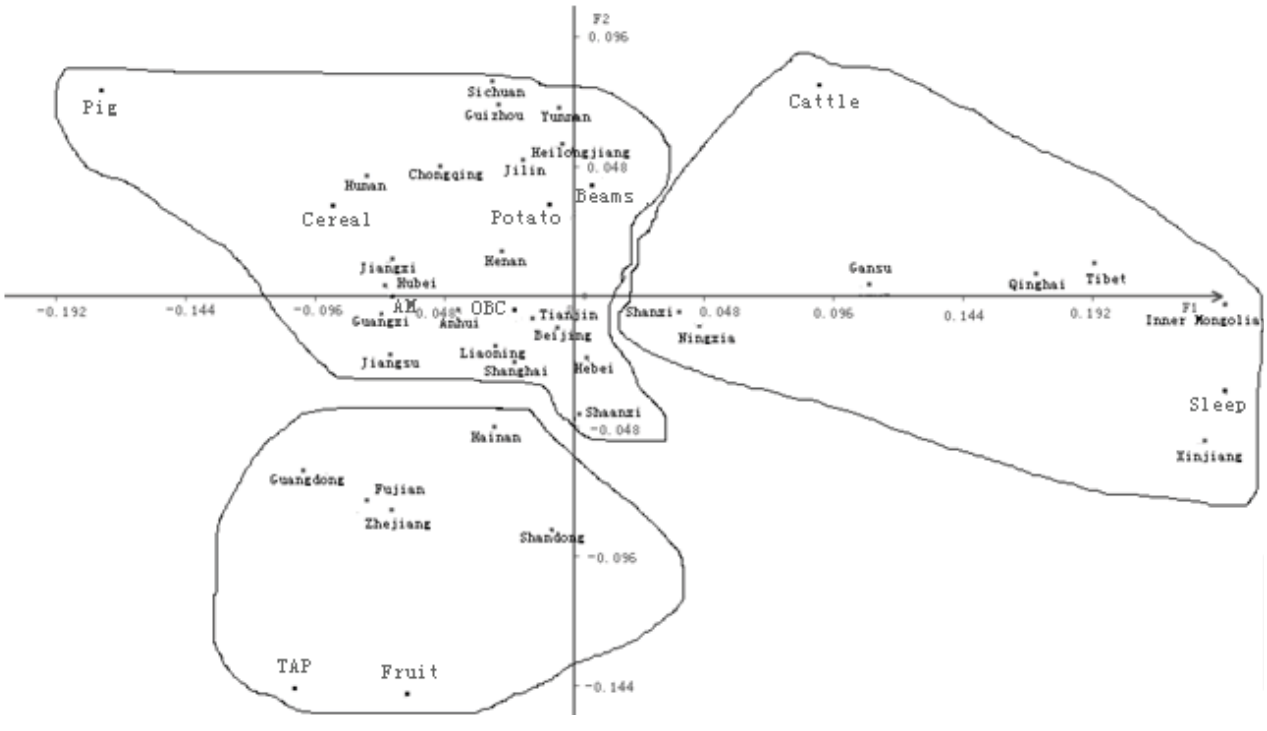

Figure 3. The scatter plot of the R-load and Q-load matrix

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Lettucenin A and Its Role against *Xanthomonas Campestris*

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Abstract

Lettucenin A is the major phytoalexin produced in lettuce after being elicited by biotic or abiotic elicitors. The production of lettucenin A in leaf can be induced by 5% of $CuSO_4$ and 1% of $AgNO_3$. A clear inhibition zone where the fungi *Aspergillus niger* failed to develop on TLC plates dipped in hexane: ethyl acetate (1:1, v/v) at R_f 0.45 was observed. Lettucenin A was detected at a retention time of approximately 5.3 min after being injected into the HPLC run with isocratic solvent system containing water: acetonitrile ratio 60:40, (v/v). *In vitro* antibacterial study with *Xanthomonas campestris* results showed this pathogen has different sensitivity to all tested concentrations of lettucenin A. The bacteria was more sensitive to higher concentration of lettucenin A (333, 533 and 667 μg ml^{-1}), compare to lower concentrations such as 67 μg ml^{-1}. Thus, the relationship between the bacteria growth rate and lettucenin A concentration was negatively correlated. However, the bacteria growth rate continues to increase after two hours of incubation. Hence, it is suggested that *X. campestris* may have the ability to detoxify lettucenin A. The success or failure of *X. campestris* to invade lettuce may very well depend on the balance between accumulation and degradation of lettucenin A at the invading sites of lettuce. In summary, lettucenin A may play an important role in the resistance of lettuce against microbial colonization.

Keywords: *Lactuca sativa*, Lettucenin A, *Xanthomonas campestris*

1. Introduction

Lettuce (*Lactuca sativa*) is a member in the Family of Compositae or Asteraceae that is grown for its leaves, which are either eaten raw or cooked (Grubben and Sukprakarn, 1994). It is a popular type of vegetable consumed in Malaysia. The production of lettuce in Sabah, Malaysia increased from year 2002 to year 2003 (Department of Agriculture 2003[a], 2003[b]) thus increasing exports from 1997 to 2000 (Jipanin et. al., 2001).

However, the growth of lettuce is always hampered by bacterial spots caused by *Xanthomonas campestris* pv. *Vitians* (Barak et. al., 2002). Plants being attacked by this pathogen developed symptoms like spots on leaves and thus decreasing its commercial value in the market. In Malaysia, farmers usually apply large quantities of chemical to control this disease, thus increasing the cost of production. Pesticides also caused contamination to soil, water and air which is hazardous to human health (Agrios, 2005; Barak et. al., 2002). New awareness to reduce the usage of chemical pesticides by developing alternative strategies or technologies in order to improve plant disease resistance and control of pathogens are being promoted.

On the other hand, there is a positive linear relationship between the amount and speed of accumulation of phytoalexin (secondary antimicrobial compound) produced and the degree of disease resistance. This relationship is also known as

quantitative relationship. When there is a higher rate of phytoalexin accumulation, the smaller lesion size and lesser number of the bacterial cells is found in the host plant (Chong et. al., 2006[a]; 2006[b]; Chong et. al., 2007[b]; Mansfield, 2000; 2007[b]). In this study, the role of lettucenin A was tested with different concentrations on *Xanthomonas campestris* to verify the antimicrobial effect of lettucenin A to *X. campestris*.

2. Materials and Methods

2.1 Plant materials and pathogens culture

Seeds of lettuce were sown in trays containing sterilized moistened soil at 25°C. Later, three weeks-old seedlings were transferred from trays to pots. Elicitations were done when the plants reached eight weeks old. *Aspergillus niger* was kindly provided by Queen Elizabeth Hospital, Sabah and maintained on Potato Dextrose Agar (PDA) at 25°C in Plant Technology Laboratory of Universiti Malaysia Sabah. *Xanthomonas campestris* was obtained from Department of Agriculture, Tuaran, Sabah and maintained on Nutrient Agar (NA) at 25°C.

2.2 Elicitation and Extraction of lettucenin A

Production of lettucenin A was induced with abiotic elicitors. Leaves of lettuce were elicited by spraying the leaves with 5 % of $CuSO_4$ or 1 % of $AgNO_3$. The leaves were incubated at 25°C for three days and then subjected to extraction by methanol 60% overnight. Extracts were filtered through Whatman No. 1 filter paper, pooled and evaporated with a rotary evaporation at 40-45°C. Homogenates were re-extracted three times with chloroform, pooled and reduced to one ml.

2.3 Detection of lettucenin A and TLC Bioassays

Thin Layer Chromatography (TLC) plates (Merck Kieselgel 60 F_{254} silica gel) were used throughout this study to detect the presence of antifungal properties of the leaf extracts and to separate lettucenin A from the crude extracts. The solvent system used was hexane: ethyl acetate (1:1, v/v). Lettucenin A gives off a bright yellow florescence when examined under UV radiation with wavelength peak of 365nm. Chromatograms were sprayed with the conidia suspensions of *Aspergillus niger* in potato dextrose broth for bioassays. Replicated chromatogram plates were prepared and sprayed with 2,4-dinitrophenylhydrazine reagent (2,4-DNPH), which gave a pink coloration after reacting with lettucenin A. Retardation value (R_f) for all bioactive and reactive bands were calculated and recorded.

2.4 Isolation of Lettucenin A

The confirmation of lettucenin A was based on the retention time (R_t) and UV absorption spectrum using High Performance Liquid Chromatography (HPLC) as described by Bennett et al., 1994. 10 µl and 50 µl of lettucenin A were injected into HPLC (Perkin Elmer Series 200), with SUPERCOCIL™ LC-18 Analytical Column, 4.6 mm x 250 mm, 5µm. The presence of lettucenin A was analyzed using isocratic solvent system 60: 40 (v/v) water: acetonitrile, running for 15 min at 25 °C with a flow rate of 1.0 ml min^{-1}. The retention time (R_t) for lettucenin A was recorded and compared with the R_t as described by Bennett et. al., 1994.

2.5 Quantification of lettucenin A

From the replicated TLC plate, fluorescing band containing lettucenin A which had the same Rf value with the inhibition zone was marked by pencil, scraped out and eluted with 100% methanol. The tubes were spun at 12,000 rpm using a centrifudge for five minutes. Supernatant containing lettucenin A was collected and subjected to spectrophotometry and HPLC. For estimation of lettucenin A concentration, a Cary 50 Bio UV-Visible Spectrophotometer was used and set to 446 nm wavelength, which is the maximum wavelength absorbance of lettucenin A (Takasugi et. al., 1985).

2.6 Antibacterial activity of lettucenin A

Different concentrations of lettucenin A (67, 200, 333, 533 and 667 µg ml^{-1}), based on the amount recovered from TLC plates were prepared. Single colony of *Xanthomonas campestris* was transferred into petri dishes containing Nutrient Broth (NB). Initial optical density (OD) of the bacteria was measured by spectrophotometer at 600 nm. The bacteria were incubated until the OD_{600} reading reached the range of 0.4-0.6 (Bacteria was in exponential stage). The bacteria within this range was tested throughout the study with different concentrations of lettucenin A in separate flask, and shaked at 220 rpm using a rotary shaker. One ml of each different lettucenin A concentration was added into each flask with three replicates for each concentration tested. After incubated for half an hour, OD_{600} for each flask was measured and the procedures were repeated for the next one hour, 1.5 hours, two hours, 2.5 hours and three hours of incubation period. For control, lettucenin A was replaced by distilled water.

3. Results and discussion

3.1 Detection of lettucenin A

Lettucenin A accumulated after elicitation with $CuSO_4$ and $AgNO_3$, and it activities against *Xanthomonas campestris*, were studied. Lettucenin A gave a bright yellow fluorescence under UV radiation. In assessments of antifungal activity,

lettucenin A was proven to inhibit the growth of *Aspergillus niger* at R_f 0.45. In addition, there were two other additional inhibition zones with the retention factor R_f 0.80 and 0.90. Nevertheless, small and slightly inhibition zone was also observed in the control (Figure 1 (b)). Identification of lettucenin A was double confirmed after sprayed with the reagent 2,4-dinitrophenylhydrazine (2,4-DNPH). Lettucenin A displayed a pink coloration after sprayed with this reagent as illustrated in Figure 2. These bands had the retention value of 0.41 and they were not significantly different compared to the retention value that gave bright yellow fluorescence and strong antifungal activity in figure 1. Thus, this compound has been subsequently confirmed as lettucenin A. UV-spectrophotometer was used to scan the maximum absorbance. The maximum absorbance of lettucenin A in methanolic solution is at 446 nm (Figure 3) as described by Takasugi et al., 1985. The presence of lettucenin A was confirmed with HPLC based on the retention time (R_t) as described by Bennett et al. 1994. Test was repeated twice with two different injection volumes, 10 µl and 50 µl. Both chromatograms had sharp peaks at R_t approximately 5.3 min although the second injection volume was five fold higher (Figure 4). An arrow indicated that the peaks correspond to lettucenin A.

3.2 Antibacterial Activity of Lettucenin A

X. campestris showed different responses after exposure to lettucenin A. Bacteria growth rates were significant lower for those exposed to lettucenin A in comparison to the control experiment. *X. campestris* were sensitive to all concentrations of lettucenin A, at least, at the first 1.5 hours of incubation period. However, growth rates of treated bacteria were not significantly different compared to the control experiment when the duration of the experiments was extended to 2.0, 2.5 and 3.0 hours incubation period (Figure 5). For bacteria treated with 67 µg ml^{-1} of lettucenin A, the growth rate was significantly lower in comparison to the control experiment at 0.5 and 1.5 hours, where the growth rate was 0.157 and 0.192, respectively. For other concentrations, the bacteria growth rate was significantly lower compared to control experiment. However, the growth rates of treated with lettucenin A were not significant in comparison to the control experiment at 2.0, 2.5 and 3.0 hours of incubation period, except for concentration of 333, 533 and 667 µg ml^{-1} at 2.5 hours.

In vitro study showed a negative correlation between the concentrations of lettucenin A and the growth rate of pathogen *X. campestris*. Higher concentration of lettucenin A had a better inhibitory effect to the growth of *X. campestris*. But the bacteria growth was also inhibited in the lowest concentration tested; 67 µg ml^{-1}. However, growth of *X. campestris* was more effectively restricted by higher concentration of lettucenin A (333, 533 and 667 µg ml^{-1}). The effect of lettucenin A against bacteria at the concentration of 333, 533 and 667 µg ml^{-1} was not significant among each other.

In other words, concentration of 333 µg ml^{-1} maybe strong enough to restrict the bacteria growth. In most cases, higher concentrations of lettucenin A (333, 533 and 667 µg ml^{-1}) were more effective against the bacteria. Different concentrations of lettucenin A had different effects on the growth of *X. campestris*. Thus, differences in the resistance and susceptibility of lettuce to *X. campestris* may associate with different concentrations of lettucenin A accumulated, as well as the speed of accumulation around the invading tissue. Localization of lettucenin A where this compound was concentrated in dead and infection sites provides good evidence in indicating the role of lettucenin A against pathogen *X. campestris*.

Besides that, the onset of hypersensitive reaction (HR) is always associated with rapid phytoalexins production. Phytoalexins were found localized to cells that had undergone HR. Fungal or bacterial invasion are then restricted in the HR cells. Accumulation of phytoalexins at the right time and place is very important parameter to cause cessation and restriction to microbial growth. After two hours of incubation, the growth of bacteria was not significant to control. This phenomenon occurred most probably because *X. campestris* may have the ability to detoxify the lettucenin A after the first 1.5 hours. Lettucenin A may be degraded by the bacteria into less toxic compounds. The ability of the bacteria to detoxify host's phytoalexin is an important determinant of pathogenicity (Kuc, 1995; Mansfield, 2000; Purkayastha, 1995; vanEtten et. al., 1989). In other words, the antibacterial action of lettucenin A is considered as bacteriostatic.

Other examples of phytoalexin detoxification were proposed in the *in vitro* relationship of *Botrytis cineria, B. fabae* with metabolism of wyerone, wyerone acid and wyerone epoxide of broad bean (*Vicia faba*). Detoxification of wyerone and wyerone epoxide was founded preceding the onset of germ-tube growth of both *B. cineria* and *B. fabae*; whereas production of secondary germ tubes from surviving conidia and sub-apical was occurred without comparable metabolism of wyerone acid. The concentration of wyerone was unlikely to decrease during the fungal-phytoalexin interaction (Rossall and Mansfield, 1984; Rossall et. al., 1980)).

Detoxification of phytoalexin by bacteria was also described in the *in vivo* relationship between cotton (*Gossypium* spp.) and bacteria *Xanthomonas campestris* pv. *malvacearum*. Cotton phytoalexins, 2,7-dihydroxycadalene (DHC) was decomposed to lacinilene C (LC) while desoxyhemigossypol (dHG) was decomposed to hemigossypol (HG). Inhibition or toxicity occurred only for the first two hours of exposure to the phytoalexins. Growth rates of *X. campestris* pv. *malvacearum* were similar to the control in the following incubation period (Abraham et. al., 1999).

The amount of phytoalexin accumulated depends on the rate and duration of phytoalexin synthesis, which will be affected by the speed where cells are killed by the invasion pathogens and the ability to tolerate and degrade the phytoalexin (Mansfield, 2000). Thus, *in vivo*, the invasion of lettuce by *X. campestris* is regulated by the balance between rates of synthesis of lettucenin A and the accumulation in the plant and the degradation of that compound by the bacteria within infection tissues (Bennett et. al., 1994). Low concentration of lettucenin A would be produced at the beginning of the interaction. If *X. campestris* is tolerant and possess the enzymic capacity to detoxify lettucenin A, it will able to grow and detoxify higher concentration of lettucenin A and kill more cells. In this case, *X. campestris* is said to be pathogenic and the invaded lettuce is susceptible to it. If the bacterium is sensitive to lettucenin A, higher concentration of lettucenin A may produce at the infection site and thus inhibiting the further invasion of the bacterium. In this case, *X. campestris* is said to be non-pathogenic and the lettuce is tolerant to it (Bennett et. al., 1994).

4. Conclusion

In vitro activity showed that the bacteria was more sensitive to higher concentration of lettucenin A such as 333, 533 or 667 μg ml^{-1} than lower concentration such as 67 μg ml^{-1}. There was a negative correlation between the concentrations of lettucenin A and the growth rate of the bacteria. However, after two hours of incubation period, the bacteria is suspected to have the ability to detoxify lettucenin A because the growth of bacteria was continuous. Lettucenin A is believed to have a role in the resistance of lettuce to microbial colonization in a sufficient concentration and at the right time. However, the exact concentration of lettucenin A that would inhibit the growth of pathogen *Xanthomonas campestris* under natural condition needs further study.

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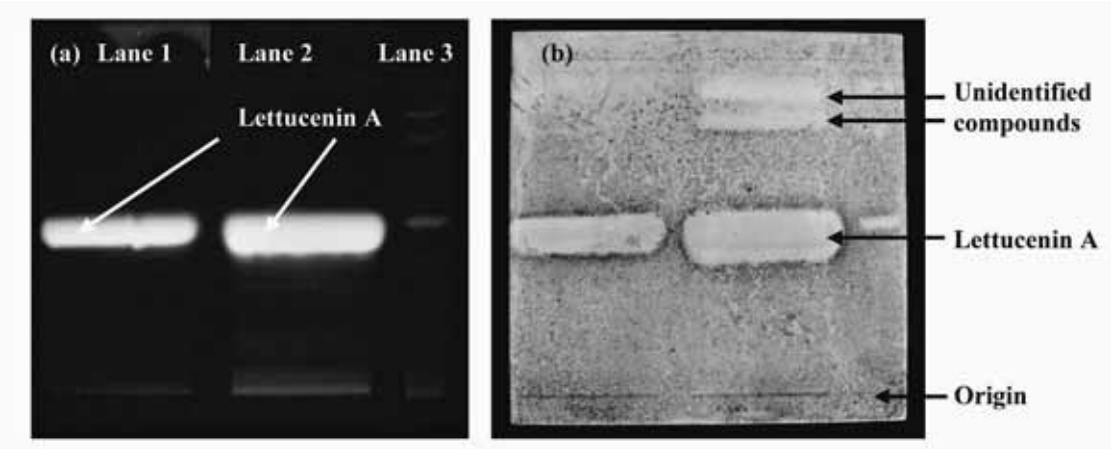

Figure 1. Chromatograms of leaf extracts developed in hexane: ethyl acetate (1:1, v/v) as the solvent system. Lane 1: lettuce elicited with 5% $CuSO_4$; Lane 2: lettuce elicited with 1% $AgNO_3$ and Lane 3: control.

(a) Observation under UV 365 nm wavelength (b) bioassay with *Aspergillus niger.*

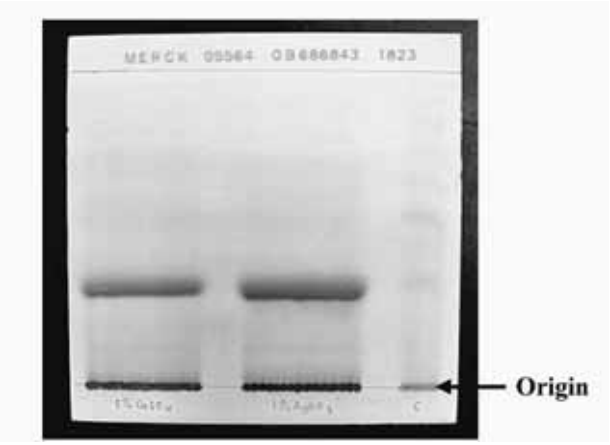

Figure 2. Chromatogram of lettuce extracts after elicited with (a) 5% of $CuSO_4$ (w/v) and (b) 1% $AgNO_3$ and sprayed with reagent 2,4-dinitrophenylhydrazine (2,4-DNPH).

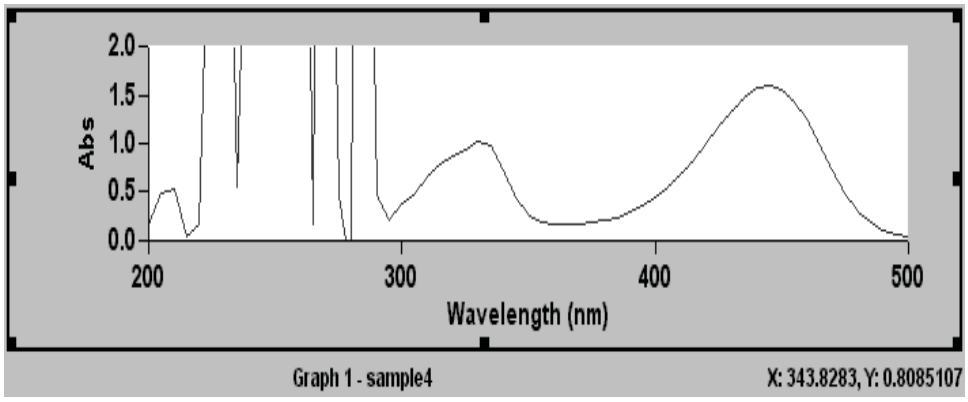

Figure 3. Ultraviolet absorption spectrum of lettucenin A range from 200-500 nm wavelengths in methanolic solution.

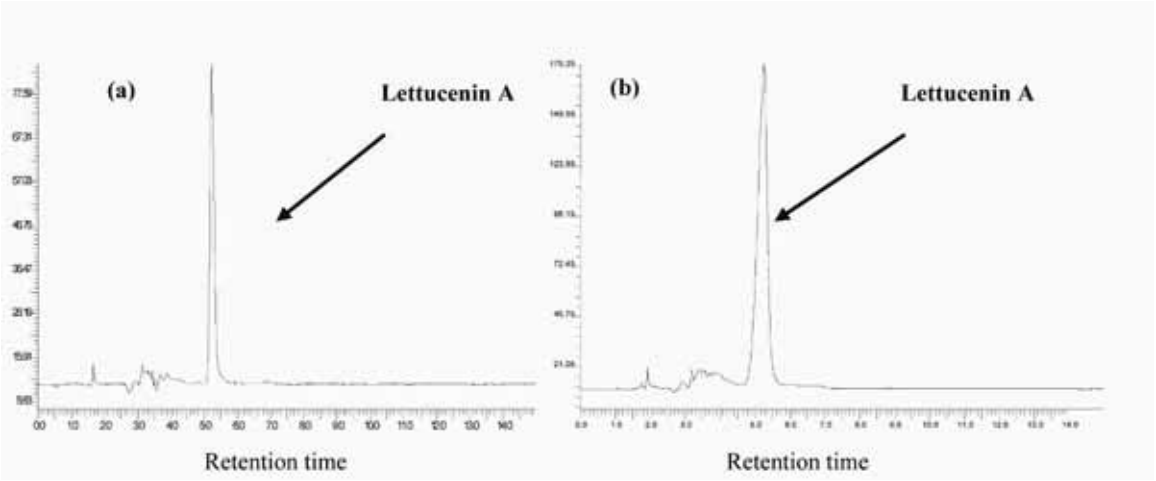

Figure 4. HPLC chromatograms at 446 nm excitation showing the presence of lettucenin A in (a) 10 µl and (b) 50 µl volume of injection.

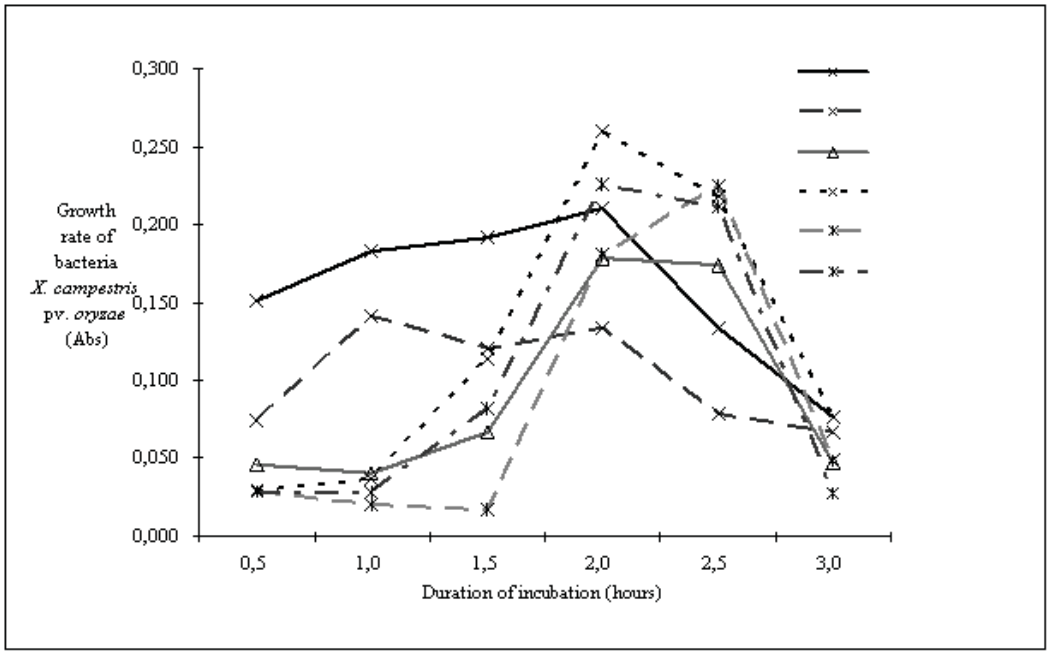

Figure 5. Comparison of growth rate of *X. campestris* in five different concentrations of lettucenin A for three hours of incubation.

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Study on Extraction and Purification Process of Capsicum Red Pigment

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Abstract

Capsicum red pigment extracted from the dry pepper is a kind of high-quality natural dye which has anticancer and cosmetic properties. First, Qiu North spicy was selected as the experimental objects by comparising the peel meal rate and relative amount of pigment of Fructus capsici, Qian chilli 2, and Qiu North spicy. Then, before extracting paprika dye, sodium hydroxide was used to eliminate the piquancy. Capsicum red pigment was extracted in Soxhlet extractor with 95% ethanol. The elution condition of column chromatographic separation was obtained by thin-layer chromatography, and the purity of capsicum red pigment was identified by methods of infrared spectrum(IR). Finally, the color value of capsicum red pigment was determined by spectrophotometer with 460nm. Results showed that the optimal process: Concentration of peppery removal agent (sodium hydroxide) was 10%, the holding temperature was 80℃, solid-liquid ratio was 1:20 (g/ml), the extraction time was 120min and the times of extraction were twice. Eluent of silica gel column chromatography separation consisted of petroleum ether and 90% ethanol with the volume ratio of 2:1. The color value of capsicum red pigment ($E_{1cm}^{1\%}$460nm) could reach to 125 and 2.88% of yield could be gained. The process was good to extract and purify capsicum red dye whose stability was high at neutral and weak acid solution.

Keywords: Capsicum red pigment, Extraction, Purification, Process, Stability

1. Introduction

Recently, the harm of synthetic pigments to human is becoming noticeable, and some of them which are even teratogenic and carcinogenic and induce chromosome variation have been forbidden using. Natural dyes have many excellent properties such as little side effect, high safety factor, biodegradable, green environmental protective. Some natural dyes have certain therapy of effect and health function. At present, researchers paid increasing attention to natural dyes and obtained some infusive findings.

Ripe fruits of red pepper (Capsicum annuum L.) are widely consumed as vegetables and are used as food colorants because they are a good source of the red carotenoids capsicum red pigment and capsorubin. Capsicum red pigment accounts for 30–60% of total carotenoids in fully ripe fruits. It contains 11 conjugated double bonds, a conjugated keto group, and a cyclopentane ring, and has stronger antioxidative effects than β-carotene. These structural characteristics give rise to singlet oxygen-quenching ability and prevent colon carcinogenesis.

To explore the optimal extraction and purification process of capsicum red pigment from dry pepper, this experiment selected as the experimental objects, and studied and analyzed its preparation process. We also analyzed the pH stability (acidic and basic stability) of the pigment puried.

2. Experimental

2.1 Materials

Pepper: Fructus capsici, Qian chilli 2, Qiu North spicy

Reagents: Ethanol (analytically pure, Tianjin Kermel Chemical Reagent Co, Ltd.), Sodium hydroxide(analytically pure, Tianjin Kermel Chemical Reagent Co, Ltd.), Hydrochloride (analytically pure, Tianjin Reagent Factory), Leveling

agent FFA, Penetrating agent JFC.

Instruments: Soxhlet extractor, High-speed universal pulverizer (FW-80, Tianjin Taisite Instruments Co, Ltd.), Electric blastdrying oven (DL-101-1B, Tianjin Zhonghuan experiment electric stove Co, Ltd.), temperature adjusting electric heating-jacket (ZDHW, Huanghua ZTE Instrument Co, Ltd.), thermostat water bath cauldron (Tianjin Zhonghuan experiment electric stove Co, Ltd.), Electronic balance (LD, Shenyang Dragon Electronics Co. Ltd.), Visible spectrophotometer (VIS-723, Shanghai Jingke Industral Co, Ltd.), Electric thermal-vacuum drying oven (DZG-04, Tianjin Tianyu Experimental Instrument Co, Ltd.).

2.2 Preparation of dry capsicum powder

Before experiment, three kinds of dry capsicum must be removed seeds and stalks and washed using tap water twice to three times. They were put into electric blastdrying oven maintaining 60℃ for 2hs and milled into powder. Subsequently, the dry capsicum powder was sift through 10-20 meshes.

A gram of dry capsicum powder was mixed with sodium hydroxide to eliminate the piquancy in the beaker, the concentration of which was 5%, 10%, 15%, 20%, 30%, respectively. Finally, dry capsicum powder eliminated the piquancy was obtained after vacuum filtrating and drying in the Electric thermal-vacuum drying oven.

2.3 Extraction of capsicum red pigment

Dry capsicum powder was transferred to Soxhlet extractor which was added into 95% ethanol. To ensure the optimal extraction process, the temperature was maintained at 30℃, 40℃, 50℃, 60℃, 70℃, 80℃, 90℃, 100℃, respectively; solid-liquid ratio was 1:5, 1:10, 1:15, 1:20, 1:25, 1:30, 1:35, respectively; time was 30min, 60min, 90min, 100min, 110min, 120min, 150min respectively and reflux times were once, twice, third, four, five, six times. Finally, head product was obtained after vacuum distillation and recovering ethanol.

2.4 Purity of capsicum red pigment

Silica gel column chromatography was selected to purify capsicum red pigment: elution condition was ensured by thin layer chromatography. First, concentration extracted was injected into the column with 100cm in high, 10cm in diameter and was eluted by eluent selected. Afterward, several parts of solution were gained and red part was collected and vacuum-concentrated to relatively pure capsicum red pigment. At last, the capsicum red pigment was analyzed by methods of infrared spectrum (IR).

2.5 Elemental analysis

2.5.1 Measurement of color value

A certain quality sample (accurate to 0.0002g) weighted was diluted with acetone, and using colorimetric utensil of 1cm thickness to measure it with spectrophotometer where the wavelength (λ) is 460nm. When the dilution multiple falls in a certain range, the wavelength is proportional to the content of capsicum red pigment. The color value was expressed as followed:

$$E_{1cm}^{1\%}460nm = \frac{Af \times 1\%}{m}$$

Where, $E_{1cm}^{1\%}460nm$ is the color value measured under the conditions of mass fraction of 1%, colorimetric utensil of 1cm, maximum absorption peak of 460nm, A is absorbance of measured sample, f is dilution multiple, and m is mass of sample.

2.5.2 Determination of yield

yield (%) = volume of capsicum red pigment (ml) / total mass of capsicum powder (g) ×100%

2.5.3 pH stability

First, capsicum red pigment was divided into ten portions adjusted to represent the pH from 1 to 10, respectively. Finally, absorbances (λ) of those ten portions were measured.

3. Results and discussion

3.1 Selection of species of pepper

Capsicum red pigment mainly exists in peel of pepper, so the larger peel meal rate is, the better effect of extraction becomes. Moreover, the larger relative amount of pigment is, the better effect of extraction becomes. In the same condition, the peel meal rate and the relative amount of pigment for three specie of pepper were showed on tab.1

Tab.1 shows that, the maximum peel meal rate is Qiu North spicy (61.2%), and the minimum is Qian chilli 2 (50.4%); the maximum relative amount of pigment is Qiu North spicy (14.62), and the minimum Fructus capsici is (7.32). Comprehensive consideration on the factor of peel meal rate and relative amount of pigment, the optimal raw material is Qiu North spicy.

3.2 Selection of the concentration of peppery removal agent (sodium hydroxide)

From observing the color change of sodium hydroxide mixed with dry capsicum powder, the peppery removal effect is good when the concentration of sodium hydroxide was 10%, 15%, 20%, 30%. However, for the concentration of 15%, 20%, 30%, the solution appeared turbid and caking during the holding process. So we selected the optimal concentration of sodium hydroxide is 10%.

3.3 Selection of extraction temperature

Before extracted, capsicum red pigment exists in cell tissue. Capsicum red pigment is protected by membrane and some components in cell tissue to form lipid, so it has high stability. Since capsicum red pigment extracted loses the protection from membrane, self-oxidation occurs easily, particularly in High-Temperature condition, color of solution fades obviously. The effect of temperature is the primary considered factor. The larger relative quantity of solvent and the longer extraction time is, the better extraction effect is. So less solid-liquid ratio and longer extraction time is the selection in this part.

Experimental Conditions is listed as follow: solid-liquid ratio is 1:35, the extraction time is 160min and the time is once. Absorbance (λ) measured in different temperature is showed in Fig.1.

Fig.1 shows that, with extraction temperature increasing, the absorbance (λ) increases gradually and reaches the peak at extraction temperature of 80℃, then it goes down. When extraction temperature at the range from 0℃ to 80℃, the increase of temperature promotes the dissolution of capsicum red pigment, when extraction temperature exceeds 80℃, the increase of temperature turns to damage capsicum red pigment for self-oxidation as introduced above. The optimal extraction temperature is 80℃.

3.4 Selection of solid-liquid ratio

Experimental Conditions is listed as follow: the extraction temperature is 80℃, the time is 160min and the time is once. Absorbance (λ) measured in different solid-liquid ratio is showed in Fig.2.

Fig.2 shows that, with solid-liquid ratio increasing, the absorbance (λ) increases significantly and reaches the peak at solid-liquid ratio of 1:20, then it changes little and basically maintaines at about 8.0-8.5. When extraction temperature at the range from 1:5 to 1:20, the solubility is the main factor of extraction effect, when extraction temperature exceeds 1:20, the solubility is not the main factor of extraction effect in that capsicum red pigment has been dissolved completely. The optimal extraction temperature is 1:20.

3.5 Selection of extraction time

Experimental Conditions is as the following: the extraction temperature is 80℃, solid-liquid ratio is 1:20 and the times are once. Absorbance (λ) is measured in different extraction time is showed in Fig.3.

Fig.3 shows that, with extraction time increasing, the absorbance (λ) increases gradually and reaches the peak (0.587) at extraction time of 120min, then it goes down. When extraction time at the range from 30min to 120min, the increase of time promotes the dissolution of capsicum red pigment, when extraction time exceeds 120min, the increase of time turns to damage capsicum red pigment for self-oxidation in High-Temperature. The optimal extraction time is 120min.

3.6 Selection of extraction times

Experimental Conditions is as the following: the extraction temperature is 80℃, solid-liquid ratio is 1:20 and the extraction time is 120min. Absorbance(λ) is measured in different extraction times is showed in Fig.4.

Fig.4 shows that, capsicum red pigment has been extracted completely after twice extraction, and absorbance (λ) is not significant change and basically maintains at about 1.01-1.02. Considering the energy efficiency, the optimal extraction time is twice.

3.7 The purification of capsicum red pigment

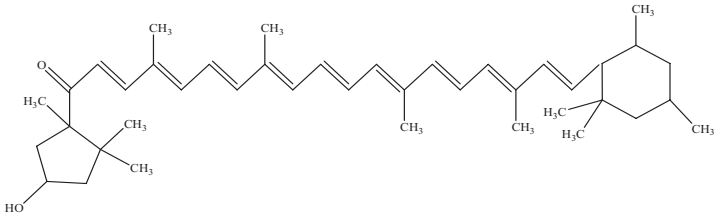
3.7.1 The selection of elution condition

Thin layer chromatography (TLC) was used to ensure the elution condition of column chromatographic separation. Comprehensive consideration on the factor of solubility, affinity, resolution and the elution curve of petroleum ether, ethyl acetate, ethanol and n-hexane, the results showed that the effect of exhibition layer is the best with petroleum ether-90% ethanol (2:1) as developer. The optimal eluent of silica gel column chromatography separation is petroleum

ether-90% ethanol (2:1).

3.7.2 Analysis on purity

The structure molecular formula of capsicum red pigment is showed as follows:



The characteristic absorption peak of Pure capsicum red pigment is as follows: the characteristic absorption peak of cyclopentane and cyclohexane is at 2950-2800cm^{-1}, the stretching vibration absorption peak of carbonyl(C=O) is at 1720-1710cm^{-1}, the scissors vibration absorption peak of methylene(CH_2) and anti symmetric deformation absorption peak of methyl (CH_3) is at 1465±20cm^{-1}, the stretching vibration absorption peak of methoxy(C-O) is at 1170-1150cm^{-1} [10]. Fig.5 represents the infrared spectrum of capsicum red dye prepared, and shows the infrared absorption band of capsicum red pigment extracted and purified is consistent with the characteristic absorption peak of Pure capsicum red pigment. Capsicum red pigment extracted and purified in this experiment has high purity and meets the need of staining.

3.8 pH stability

First, the pigment sample is diluted with the concentration 1:200, and then is divided into ten portions adjusted to represent the pH from 1 to 10, respectively. Finally, absorbances (λ) of those ten portions are measured and showed in Tab. 2.

Tab.2 shows that, when pH is at the range of 1-2, a slight increase of pH will bring a significant increase in absorbance (λ) and when pH is at the range of 6-7, a slight decrease of pH will bring a significant decrease in absorbance (λ), and when pH is at the range of 2-6 and 7-10, the alter of absorbance (λ) is not noticeable with the increase of pH. It is clear that capsicum red pigment can be damaged in strong acid or alkaline condition, and keep relatively stable at neutral and weak acid solution.

4. Conclusion

Results showed that the optimal process was listed as follow: the raw material was Qiu North spicy, concentration of peppery removal agent (sodium hydroxide) was 10%, the extraction temperature was 80℃, solid-liquid ratio was 1:20 (g/ml), the extraction time was 120min and the time of extraction was twice, eluent of silica gel column chromatography separation was petroleum ether-90% ethanol (2:1). The color value of capsicum red pigment (E1cm1% 460nm)could reach to 125 and 2.88% of yield could be gained. The infrared absorption band of capsicum red pigment extracted and purified is consistent with the characteristic absorption peak of Pure capsicum red pigment. The process was good to extract and purify capsicum red pigment and the stability of which was high at neutral and weak acid solution.

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Table 1. Property of different species of pepper

Species	*Dry* capsicum weight (g)	Peel meal weight (g)	Peel meal rate (%)	Absorbance	Relative amount of pigment
Fructus capsici	250	141.2	59.3	0.387	7.32
Qian chilli 2	250	125.1	50.4	0.452	8.64
Qiu North spicy	250	150.5	61.2	0.683	14.62

Table 2. Stability influence of different pH Values

pH	1	2	3	4	5	6	7	8	9	10
λ	0.057	0.535	0.448	0.458	0.564	0.629	0.091	0.077	0.221	0.046

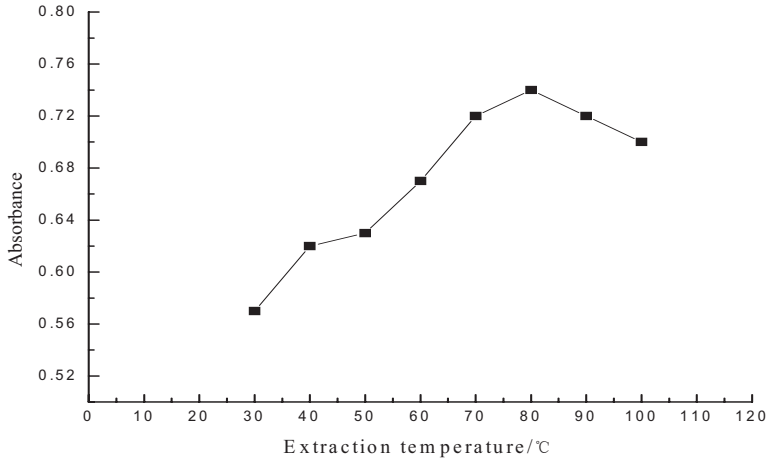

Figure 1. Extraction effect of extraction temperature

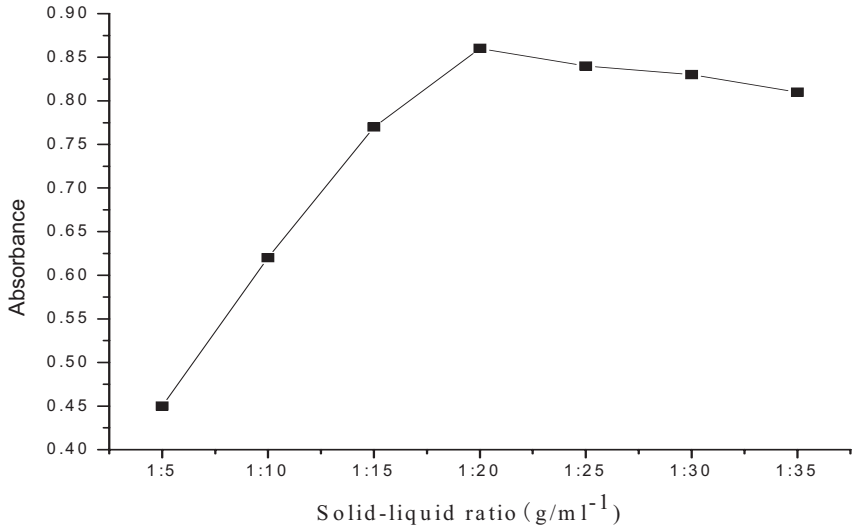

Figure 2. Extraction effect of solid-liquid ratio

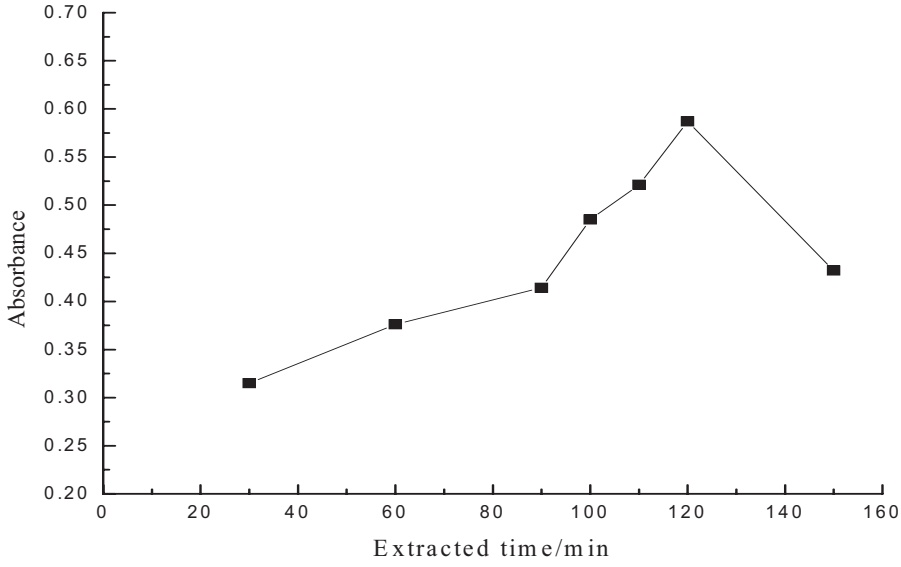

Figure 3. Extraction effect of extraction time

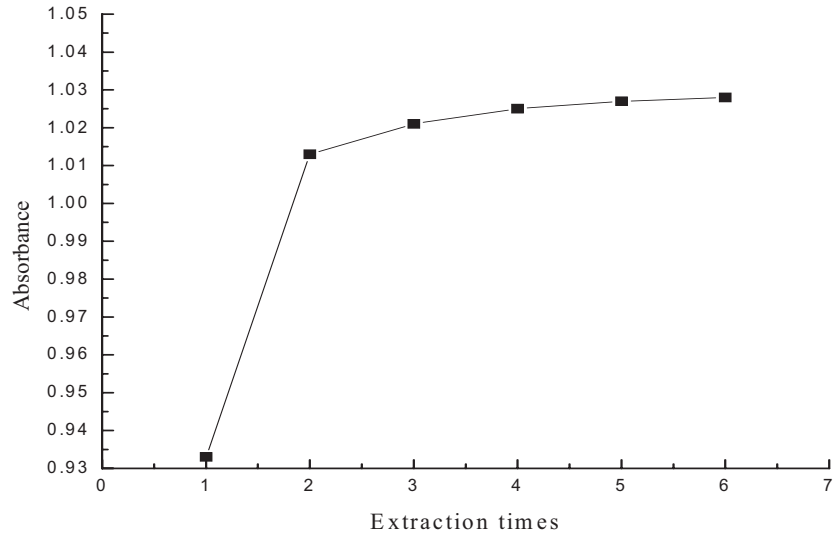

Figure 4. Extraction effect of the extraction times

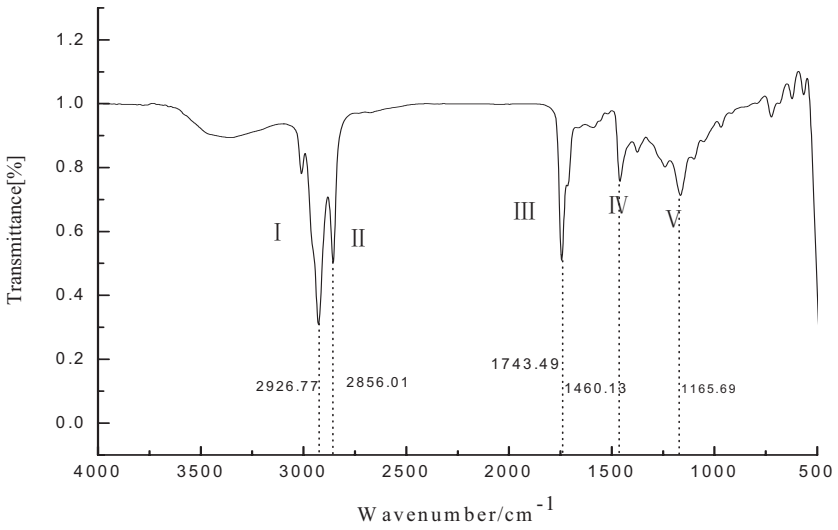

Figure 5. Infrared spectrum of capsicum red pigment

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A Microcontroller-Based Monitoring System for Batch Tea Dryer

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Abstract

This paper presents an automated tea dryer system based on programmable controller which controls moisture content of the tea leaves and temperature of the chamber in different stages of drying. Several techniques were used for tea drying systems according to the tea genres. The batch tea dryer is designed with 6 to 8 trays. The temperature above the trays is controlled between 50ºC and 100ºC. Moreover, the moisture content of the tea leaves declined from around 68% to approximately under 3%. In addition, the temperature of the leaves increased from a little less than 30ºC to 80ºC. A microcontroller as the main processor was deployed to process received data from sensors and also it provides control signals. Thus, this system equipped a data logger memory to record data during drying process. The analyses of dryer products shown the feasibility of using propose system for batch tea drying.

Keywords: Tea dryer, Batch, Moisture, Microcontroller

1. Introduction

In manufacturing of black tea the moisture of the tea (termed dhool) is about 70% wet bases (W.B.) is decreased to target moisture of 2.5% - 3% W.B. in approximately 20 minutes period in order to obtain the best quality of the product (Temple, 2001).

Although in tea production the most energy consuming process is drying, but because tea manufacture is mainly in countries that labor costs are low, automation and monitoring and process control were not an issue in the past. For this reason most factories process control is done manually.

Typical dryer capacity fluctuate from about 100kg/hr to 300kg/hr and the average of fuel consumption is around 45kg/hr. Absence of controlling will lead to wastage of energy and moreover to lower throughputs.

Nowadays, producers know the importance of process control which will lead to increasing quality but investing in high technology equipments is not always able to be carried out. Therefore, automation equipment is better to be

manufactured locally. Tea drying process is used to be concerned with monitoring and detecting the inlet air temperature, outlet air temperature, drying time, air volume and feed teas which differ in their moisture contents and grade.

In tea drying different types of dryers are applied to reduce the moisture content of the wet dhool. The most regular dryer is the fluid bed dryer followed by an endless chain pressure dryer. Even though it has its advantages but it is rarely encountered and cross flow dryer is not normally used because of sensitivity to changes in feed rate. Predictably, this type is highly sensitive to load variation, could similarly be susceptible to changes in hot air temperature and flow rate. Ultimately modifications would affect the process (Temple, 2000).

In tea drying different types of dryers are applied to reduce the moisture content of the wet dhool. The most regular dryer is the fluid bed dryer followed by an endless chain pressure dryer. Even though it has its advantages but it is rarely encountered and cross flow dryer is not normally used because of sensitivity to changes in feed rate. Predictably, this type is highly sensitive to load variation, could similarly be susceptible to changes in hot air temperature and flow rate. Ultimately, modifications would affect the process (Temple, 2000).

The automated controllers are employed to monitor drying process systems. Several monitoring systems are used in industrial companies such as programmable logic controller (PLC). The installing of these systems is very expensive and required a large floor area. In the small-scale systems such as proposed dryer, the automation with PLC is not commercial. Therefore, the low-cost approaches are required to manufacturing the small-scale systems. One of these approaches which is utilized in our system is microcontroller-based hardware. This module is used in proposed dryer as the main processor.

The objective of this work is to design a batch tea dryer with controllers. The proposed tea dryer is designed for effective control of the drying process is implemented with microcontroller.

The variables that were investigated were the objective of drying, model of batch dryer, data logging system and control strategy based on microcontrollers.

2. Tea Drying Method

The drying process occurred in a closed chamber where hot air is passed over tea leaves which resulted to heat and mass transfer between air and the drying leaves. Energy is provided by air stream to supply the heat needed for evaporation of moisture from tea, the evaporated moisture is carried away from the tea leaves by air stream (Jayaraman, Gupta, 1995).

The cycle of air drying because of the high moisture content in tea includes three stages, the equilibrium stage which food is heated to reach its drying temperature the constant rate stage that water evaporates from a saturated surface of tea at a proportional rate to moisture content and falling rate period which begins after the moisture content is reached (Canovas, Ma, Barletta, 1997). During the constant rate period, the temperature of the surface of tea becomes equal to wet bulb temperature analogous to air temperature and humidity in similar locations. During the falling rate stage, tea temperature reaches the dry bulb temperature of the air (Mujumdar, Menon, 1995). Values that are established from moisture studies are crucial for understanding how food absorbs and loses moisture (Temple, Boxtel, 2000). In black tea manufacturing the macerated leaf undergoes fermentation which is actually an enzymatic oxidation (Temple, 2001). If the level moisture of the input tea is more, it will require more heat to remove it. The method for measuring the humidity of air at drying temperatures was with a psychrometer (Temple, Boxtel, 1999). Measuring the wet bulb and dry bulb temperature the device modifies the inlet and exhausted air. Inlet air temperature should be above 83ºC to bestow good quality of dried tea and it should be below 99ºC to prevent tealeaves from case hardening and burning.

The moisture that is in the air from the drying process is the exhausted temperature, which illustrates drying conditions. The exhaust temperature should be between 49ºC and 57ºC depending on the inlet air temperature. It is important to include that if the exhaust temperature is higher the efficiency of the dryer while be lower.

Furthermore, it is understood air acts like a medium in tea drying process. The volume of air required depends on the type of tea. Moreover it is obvious that the leaves thickness can affect the drying process if the leaves are thick they can cause backpressure. Lastly, drying time is important to obtain a stable drying. Long drying damages the tea quality and fast drying can cause the tea to be bitter. Usually tea-drying duration is between 15 and 25 minutes, which is dependent on the quantity of the heat, tea leaf thickness, and certainly on the type of tea.

3. Dryer Design

Air which is heated with gas or oil is not used due to the fact that in burning process it may make smoke which will give tea leaves a low quality. A tray or cabinet batch dryer is the simplest type of dryer (Crapiste, Rotstein, 1997).

In this dryer there is a heater device which provides the hot air with approximately 17000 cubic feet per minute (CFM) moreover it will reach the temperature around 94ºC which is required for the process. This air is sent to the chamber with an air fan. The fan pulls air horizontally between and vertically through the batch dryer trays. Via the damper exhausted air can be recycled into the chamber again. The designed dryer has stainless steel trays, which have punched holes, so the heated air could pass though them. Each batch drier is designed to carry 6 to 8 trays (Javanmard, Arvin,

2008). The spread thickness depends on the type of tea for thin leaves, it could be feed to about 5cm but for the thick leaves, it is better to use a liner layer for about 4cm. However, if the tea is placed in layers with approximately 2cm to 5cm it would be possible to detect drying conditions in a superior way.

Moreover, in this design there were a change in the position of the fan and the heater system. As it is shown in Fig.1 originally the heater is put after the fan. Reversing the position of fan and heater will affect the drying rate. Thus, this change was indicated in Fig.2.

As shown in Fig.2 (a) air is first heated and then sent into the fan. The air in this condition will have less turbulence so the fan can send the air with a higher rate in velocity, if so the evaporation of water from the tea will increase. In contrast, the high heat will damage the fan so technically it is not so good even though the rate of drying will increase. In the dryer proposed which is shown in Fig. 2 (b) the position of the fan and the heater is changed so in this case the ambient air will first pass through the fan so their would not be any damage and then it will be heated. It is for certain the drying rate will decrease but also the energy cost and damage of the fan will decrease will it too. So another important aim of the dryer which is being economic will be established.

In addition, there is another fan inserted in the dryer. The purpose of putting another fan is to get higher efficiency. Due to getting feedback's from the system continuously, if the threshold of the chamber temperature suddenly increases from the predicted temperature the second fan will start working. Thus it will cause suction, so if the hot air in the chamber to outside and the temperature will get back to the target which was mentioned previously. In this way, the product will be prevented from burning. High temperature may cause the product to lose more moisture, which is not desired. Also, losing more moisture can cause losing valuable micro components in tea and change the taste too.

4. Processing unit

In industrial high capacity, dryers use PLC-based systems in monitoring unit. These systems are very expensive and it is not suitable for small-scale systems. Another approach to solve this problem is using low-cost controllers. The Low-cost controllers require some reliable noise filtering circuits in power supply and analog sensor units. If good noise filters are selected in monitoring circuit, the suitable and also low-cost controller will be obtained. One of these approaches is the microcontroller processor. Therefore, a microcontroller as the main processor is deployed to process sensors data and manipulates suitable behavior in drying process. The designed dryer includes several electronic units, which are digital and analog components, as shown in Fig. 1. The processor unit must be able to execute several tasks such as Reading sensors' value, Manipulate suitable control signal, Fan and heater control, Data logging, PC communication.

For sensory system managing, analog to digital converter (ADC) unit of microcontroller is used to digitize captured sensor's value. This unit provides digital value of analog sensors. After capturing samples of sensors, processing function decides suitable output signals according to defined drying period. In proposed system, Fan 1 and heater unit are controlled with pulse width modulation (PWM) approach. In this technique, when the pulse width gets longer it results to increasing output values which are fan's speed and heater temperature. Table I illustrates the ratio of PWM to the output value percentage. The PWM variable is between 0 and 1023. The average of output value is calculated by the following formula:

$$V_{out} = \left(t_{on} / \left(t_{on} + t_{off}\right)\right) \cdot V_{in} \quad (1)$$

Where t_{on} is duration of pulse with logic one and t_{off} is duration of pulse with zero logic.

The PWM technique allows controlling utilized devices in various output levels. This approach provides to control heater temperature smoothly. In older drying systems, heaters and fans were controlled with relay and controller sent only ON and OFF commands to relay. Also, the Fan 1 speed is controlled with PWM signal. Two separate PWM channels are used to control heater and Fan 1.

In addition, an external memory is used to record sensors value as the data logger. This memory is structured as the simple data base which has two fields, time and sensors' value. Sampling step is changeable for each types of drying process.

The other feature of this controller is capability to communicate with PC. Two applications are defined in this connection, i) reading recorded values during drying process, ii) on-line controlling. After connecting dryer to PC, data logger values will be downloaded by standard serial cable. Downloaded values are real data in drying process to be use for drawing dryer process diagrams. In addition, designed system enables to get control signals from PC. During drying process, PC receives digitized sensors value and decides provides control commands, which are fans, heater, and valves control signals.

5. Results and Discussion

Sufficiently wet tealeaves are fed into the dryer; in this stage, wet tea is in contact with high temperature air (around 95ºC) then after tealeaves start drying. In this procedure, air takes the moisture from tea thus its dry bulb temperature declines. At the same time as the dry bulb temperature of air drops tea leaves start losing there moisture content and

there temperature increases from wet bulb temperature of air to dry bulb temperature of air. The heat utilization of the air above the trays was found when the discharging tea was at the moisture content of around 3%. The number of trays in this experiment was eight. Fig. 3 indicates the relationship between moisture content of leaves and their temperature.

As shown in Fig. 3 at the first stages, the slope decreases dramatically but when it reaches to less than 10% of moisture content, it declines slightly around 3%.It can be understood that the leaves temperature increases significantly when the leaves have lost moisture in a considerable quantity. The raise in temperature is only 10ºC from the moisture content of 68% to less than 10% but from this point further the temperature increases from around 45ºC to 80ºC. The reason for this matter is that at the begging of the process tea leaves get the temperature to reach to the critical moisture content so they use the heat that they receive from air to lower their moisture content, and then when they reach to the critical point the heat that they obtain from the air is used to increase their temperature.

Fig. 4 illustrates the relationship between moisture content of the leaves and the temperature of them in each tray by centigrade in different stages of drying. The capacity of the dryer is proportional to the heat of the air above the trays. Although, if the temperature is not high enough it could lead to liquor and stewing of the leaves during the drying process but very high temperature can also cause casehardening and burning of the leaves. The temperature of the air above the trays was between 50ºC and 95ºC so the less damage would occur on tea leaves. Moisture content of leaves dropped from 68% to under 3% at 95ºC.

Equilibrium moisture content could be found by the standard method (ISO 1980) the technique is by measuring the decrease in mass at temperature of 100ºC -105ºC, the tea equilibrium with the air at above 100ºC is determined as having zero moisture content, hence the equilibrium moisture of tea with ambient air to over 100ºC is zero(Temple, Boxtel, 1999)

5. Conclusion

The batch tea dryer system based on programmable controller is presented in this paper. We designed a new dryer machine, which utilizes hot air chamber to drying several tea genres. Pulse width modulation approach is employed to control heater and fan with smooth level. The several performed experiments show that the best quality of dried tea leaves will be occurred when the chamber temperature was above 83ºC and 99ºC. Furthermore, the exhaust temperature must be in the range of 49ºC and 57ºC that was depended on the entrance air temperature. And the leaves temperature increased from 30ºC to 80ºC. The drying process took about 15 to 25 minutes depends on the thickness of the leaves, quantity, and types of the tea which are subjected to drying. In this process, the inlet air was 94ºC and the moisture content of the leaves reached to the target of less than 3%. The proposed dryer is mostly designed for laboratory scales and the obtained results were efficient for industrial layouts.

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Table 1. The PWM variable value and output percentage

PWM Register Value	Output Percentage
0	0%
256	25%
512	50%
768	75%
1023	100%

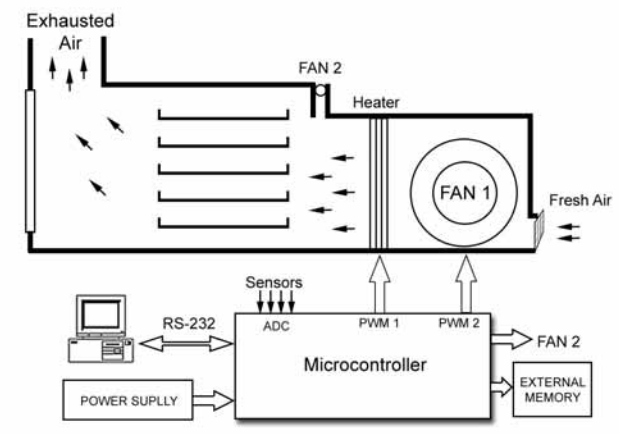

Figure 1. Schematic of designed tea dryer

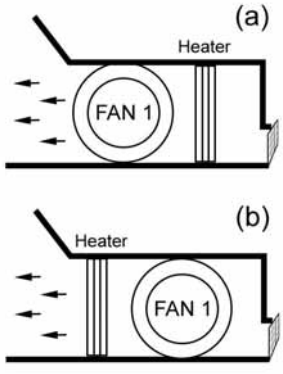

Figure 2. (a) Heater is before the fan, (b) heater is after fan

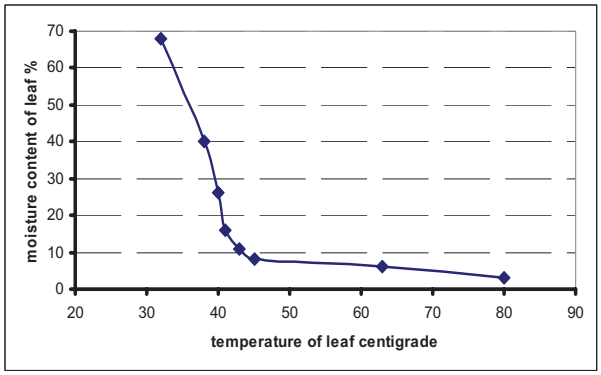

Figure 3. Temperature of leaf versus moisture content of leaves observed in drying process

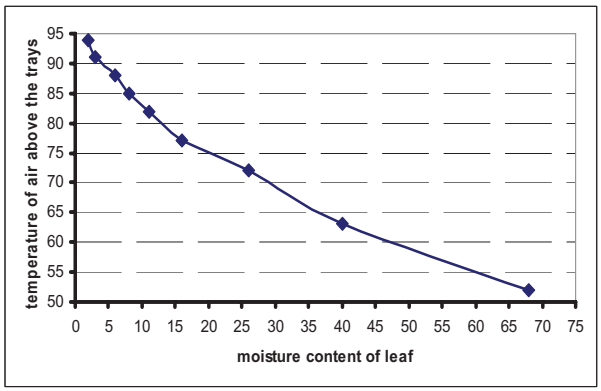

Figure 4. Temperature ratio of air above the trays versus moisture content of leaves observed in drying process

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Effect of Every-Other Furrow Irrigation on Water Use Efficiency, Starch and Protein Contents of Potato

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Abstract

The every-other furrow irrigation is one of the mothods of deficit irrigation in furrow irrigation system. In this research, a randomized complete block design with three irrigation treatment and four replication on potato was stablished in Agricultural Research Center,Shahrekord, Iran. The irrigation treatments were: normal furrow irrigation(N), fixed every-other furrow irrigation(F) and alternative(variable) every-other furrow irrigation(V). The frequency of irrigation was constant and depth of it was calculated by measurement of soil moisture deficit and the volume of irrigation water was measured by a volumetric counter. The water and soil quality was normal (EC less than 1 ds/m). The different fertilizers were used. After harvesting, water use efficiency, starch and protein content were measaured for each plot. There was significant difference between water use efficiency under different treatments, so that, the F treatment had the most water use efficiency. The every-other furrow irrigation decreased the starch content significantly. The V treatment increased the starch content significantly related to F treatment. There was no significant difference between the protein contents in the three treatments.

Keywords: Every-other furrow irrigation, Water use efficiency, Starch, Protein

1. Introduction

Potato is one of the most important and popular crops throught the word. The crop has attained great importance and popularity during the past two decades (Kashyap and Panada,2003). Potato needs frequent irrigation for its good growth and yield. The yield quality is greatly influenced by amount of applied water.(Marutani and Guz, 1989, Trebejo and Midmore,1990). Storage of water in arid and semi arid regions of iran is an important limiting factor in crop production. Deficit irrigation is one of the methods for this purpose, because it is able to increase benefit and water use efficiency. Plants with short growing season and good resistance to water stress are suitable for deficit irrigation. One of the best methods of deficit irrigation in furrow irrigation method, is every- other furrow irrigation. Each plant is irrigated by infiltration from one side of furrow in this method. This method promotes irrigation efficiency and prevents losses of water (Fischbach et al.,1974, Musick et al.,1982, Stone et al. 1993). For example, water use efficiency of sugarbeet was determined under every-other furrow irrigation in Shiraz, Iran, so that, this method increased the water use efficiency significantly (Sepaskhah et al.,1997). However, the result of New(1971) for sorghum and Samadi and Sepaskhah(1984) for dry bean indicated a significant yield reduction in every-other furrow irrigation. The reduction of yeild was due to the smaller amount of applied water and apparent imposed soil moisture stress, especially at the reproduction stage of grwth. Every0other furrow irrigation with supplemenary every-furrow irrigation at pod filling stage produced the highest bean daily with a smaller amount of water compared with the every-furrow irrigation(Samadi and Sepaskhah,1984). Crop yield may increase by using proper irrigation scheduling. Grain yeild of fingermillet was increased at more frequent irrigation intervals(Vanangamudi et al.,1990). Moorby et al.(1975) concluded that the every-other furrow irrigation method had no significant effect on the sugar and starch contents of potato. The objectives of

this research were to determine water use efficiency, starch and protein contents of potato under every-other furrow irrigation and compare with every-furrow irrigation results.

2. Materials and methods

The experiment was conducted on potato (Marfona cultivar) at center of agricultural research of Shahrekord, Iran in 2004. Some soil properties prior to planting are shown in Table 1.

The design of the experiment was a randomized complete block with four replications and consisted of three treatments of furrow irrigations: (1): every-furrow irrigation or normal method (N) in which water has been applied to every furrow ;(2): fixed every-other furrow irrigation (F) in which water has been applied as fixed every-other throughout the growth saeson ; and (3): variable or alternative every-other furrow irrigation (V) which is similar to F, but water has been applied to the furrow which was dry in the previous irrigation cycle. Therefore there was 12 plots in which there were 6 furrows with 10m long and 0.75m spacing. The irrigation interval was 7-day and amount of water for each irrigation was determined by measuring the soil water content in root zone by gypsum block before irrigation and raising the soil moisture to field capacity. The volume of irrigation water was measured by a volumetric counter and it is shown in Table 2. (Runoff was zero). According to this Table, the treatment were applied since 5^{th} irrigation. The water and soil quality was normal(EC less than 1 ds/m).

Potato were planted to a distance of about 0.3m in the rows and 0.1m deep on May 25, 2004. Nitrogen, phosphorous, potasium and micro fertilizers were applied prior to planting and during growing season base on requirement. Potato yeilds were harvested on September 26 in the same year. The yeilds were weighted, water use efficiency was determined in each plot. The starch and protein contents were measured in each plot by AOAC(Association of Official analytical Chemists. by Williams,1984) and Kejeldal method respectively.

3. Results

The average water use efficiencies(WUE) were 621, 772 and 710 grams dry mass per cubic meter and 2.87, 3.51 and 3.23 kiligrams wet mas per cubic meter for N, F and V treatments respectively(Table 3 and 4). The WUE and its analysis of variance has been manually made and shown in Table 5 and 6. According to Table 5 and 6, there is significant difference(significance level is 5%) between WUE (dry and wet) under three treatments. According to these Tables and based on mean comparison test (Duncan's test), there is no significant difference between WUE under the F and V treatments However, we can conclude that every-other furrow irrigation with normal water irrigation has a significant effect on WUE.

The average of starch contents were 66.66, 62.09 and 64.54% for N, F and V treatments respectively (Table 7) and its analysis of variance has been shown in Table 4. According to Tables 8, there is significant difference(significance level is 1%) between starch contents under the three treatments while every-other furrow irrigation method decreased the starch content significantly. According to Tables 8 and mean comparison tests, there is significant difference(significance level is 5%) between starch contents under the F and V treatments, so that the starch content in the V treatment is more than F one.

The average of protein contents were 8.41, 7.67 and 8.97% for N, F and V treatments respectively (Table 9) and its analysis of variance has been shown in Table 6. According to Tables 10 there is no significant difference between protein contents under the three treatments.

4. Discussion

The every-other furrow irrigation is one of the methods of deficit irrigation in furrow irrigation. It was concluded that the every-other furrow irrigation method increased the WUE and decreased the starch content and was no effect on protein content of potato in the studied area. There was no significant difference between fixed and alternative every-other furrow irrigation methods except for starch content,so alternative method increased starch related to fixed method. Because the starch content of potato irrigated by every-other furrow irrigation method, is suitable for nutrition and this method save the application water, so this method is proposed for the studied area. This research showed that the every-other furrow irrigation method can be saved water and increased area under cultivation about 62%(45 divided to 27.9 from last row of Table 2) and increased yield about 22% (3.51 divided to 2.87 from last row of Table 3) and 12% (3.23 divided to 2.87 from last row of Table 3) in fixed and alternative methods respectively.

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Table 1. Soil properties prior to planting

Ca+Mg (meq/l)	P (mg/kg soil)	N (%)	K (mg/kg soil)	Na (mg/kg soil)	PH	EC (ds/m)	Texture
1.7	36.96	0.066	6.9	6.1	6.49	0.19	Silty clay

Table 2. Irrigation scheduling

Irrigation No. (1)	VF (2)	VPN (3)	VPE (4)	VT (5)
1	0.55	3.3	3.3	39.6
2	0.36	2.16	2.16	25.92
3	0.41	2.46	2.46	29.52
4	0.48	2.88	2.88	34.56
5	0.56	3.36	1.68	26.88
6	0.6	3.6	1.8	28.8
7	0.58	3.48	1.74	27.84
8	0.53	3.18	1.59	25.44
9	0.51	3.06	1.53	24.48
10	0.49	2.94	1.47	23.52
11	0.48	2.88	1.44	23.04
12	0.44	2.64	1.32	21.12
13	0.4	2.4	1.2	19.2
14	0.39	2.34	1.17	18.72
15	0.38	2.28	1.14	18.24
16	0.34	2.04	1.02	16.32
Sum	-	45	27.9	403.2

(2):Volume of water entried to one furrow(cubic meter)

(3):Volume of water entried to one plot with fixed or alternative every other furrow(cubic meter)

(4):Volume of water entried to one plot with normal furrow(cubic meter)

(5):Volume of water entried to total farm(cubic meter)=(4)*8+(3)*4

Table 3. The results of WUE (based on wet mass, Kg/m^3)

Treatment Replication	N	F	V
1	2.47	3.21	3.19
2	2.98	3.26	3.08
3	2.91	3.73	3.15
4	3.13	3.84	3.48
average	2.87	3.51	3.23

Table 4. The results of WUE (based on dry mass, Kg/m^3)

Treatment Replication	N	F	V
1	499	717	722
2	599	698	654
3	629	758	691
4	758	917	774
average	621	772	710

Table 5. Analysis of variance for WUE (based on wet mass)

SV	DF	SS	MS	Fs
Block	3	0.45	0.15	4.9
Treatment	2	0.81	0.41	13.1
Error	6	0.19	0.03	
Total	11	1.45		

Table 6. Analysis of variance for WUE (based on dry mass)

SV	DF	SS	MS	Fs
Block	3	56910	18970	7.71
Treatment	2	46225	23112	9.4
Error	6	14757	2460	
Total	11	117892		

Table 7. The results of starch content(%)

Treatment Replication	N	F	V
1	64.87	61.91	64.54
2	68.74	64.3	66.05
3	67.26	60.37	64.64
4	65.76	61.76	62.93
average	66.66	62.09	64.54

Table 8. Analysis of variance for starch content

SV	DF	SS	MS	Fs
Block	3	15.52	5.17	5.16
Treatment	2	41.9	20.95	20.9
Error	6	6.01	1	
Total	11	63.43		

Table 9. The results of protein content(%)

Treatment Replication	N	F	V
1	8.81	4.39	9.14
2	7.48	9.23	8.95
3	7.97	8.36	8.97
4	9.38	8.69	8.82
average	8.41	7.67	8.97

Table 10. Analysis of variance for protein content

SV	DF	SS	MS	Fs
Block	3	3.72	1.24	0.56
Treatment	2	3.41	1.71	0.78
Error	6	13.2	2.2	
Total	11	20.3		

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Study of Integrate Methods Chemical and Cultural Control of Weeds to Wheat (*Triticum aestivum* L.)

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Aabstract

An experiment was laid out to study the effect of different weed management practices and row spacings on the yield and yield components of wheat variety Karaj-2 during 2005 – 2006 at Agronomy Research Farm, University College of Agricultural and Natural Resource, University of Tehran. Wheat was sown at row spacing of 20, 30 and 40 cm, while herbicides applied were broad spectrum 2,4-D butyl Ester: 72 EC + Isoproturon 75 WP @ 865 ml and 865 g ha-1 respectively, broad leaf Buctril M 40 EC @ 1.25 liter ha-1 and a weedy check (no herbicide). Row spacing significantly affected all parameters. Minimum weeds density m-2 and maximum spikelets spike-1, grain yield, harvest index and net income were found in 20 cm row spacing. While number of grains spike-1 were highest in 30 cm row spacing. Among herbicides, minimum weeds density m^{-2} and maximum spikelets $spike^{-1}$, grains $spike^{-1}$, grain yield, harvest index and net income were found in plots treated with broad-spectrum herbicide followed by Buctril-M 40 and the weedy check, respectively. It is concluded that maximum grain yield and net income can be obtained using broad spectrum (grasses + broad leaved) herbicides and narrow row spacing.

Keywords: Integrate methods, Herbicides, Row spacing, Wheat, *Triticum aestivum,* Yield

1. Introdution

Wheat (*Triticum aestivum* L.) is globally important cereal crop with respect to area and production. In Pakistan, it ranks first among the cereal crops and occupies about 66% of the annual food crop area (Anonymous, 2003). The area in Iran under wheat cultivation in 2005-2006 was 8.14 million hectares, producing 18.54 million tons with an average yield of 2.28 t ha^{-1} (Anonymous, 2005). The wheat yield in Iran is lower as compared to other advanced countries of the world. Cultural management plays significant role in increasing production ha^{-1}. Among which weed control, row spacing and quality seed can improve yield by about 50 – 70 percent (Ashrafi et al, 2009, Burns, 1944). Adapting the above-mentioned technology in the country we will be able to export more wheat to other countries or to allocate some area of wheat to the production of other exportable agricultural commodities. Weeds are one of the major constraints in

crop production. They compete with crop plants for light, moisture, nutrients and space. Weeds also increase harvesting costs, require costly cleaning of seeds, clog water ways, and increase fire hazards (Arnon, 1972; Ashrafi et al, 2009; Rahnavard et al, 2009). Young *et al*., (1994) reported that weeds reduced the wheat yield from 9.50 to 16.03% depending on the intensity of weeds. It is therefore, essential to control weeds in order to obtain maximum yield of wheat having good quality grains. Management of weeds has been practiced from time immemorial by manual labor or animal drawn implements. These methods, besides being laborious and tiresome are expensive due to the increase in labor, animal and implements cost (Iqbal, 1994) and as such have stimulated interest in the use of chemical weeds control. But, the exclusive reliance on herbicides has resulted in pollution of the environment and inter- and intra-specific shifts (Integrating the chemical with cultural is an excellent option for the weed control (Hassan and Marwat, 2001). Proper row spacing is another most important management factor affecting the agronomic characteristics of wheat and weed infestation (Marwat et al, 2002). Narrow row spacing produces high leaf area index, which results in more interception of photo-synthetically active radiation and dry matter accumulation (Ashrafi et al, 2009Tollenaar and Aguilera, 1992 and Dwyer et al., 1991).

A limited research has been carried out in Iran on the integrated efforts of wheat production. In order to ascertain the integrated use of crop management practices, the present study was conducted in irrigated plains of Karaj City with the objectives to evaluate the impact of integrated weed management viz. chemical and cultural on the agronomic parameters and economics of wheat production.

2. Materials and methods

A study was undertaken on wheat variety Karaj-2 at the Research Farm, Agronomy Research Farm, University College of Agricultural and Natural Resource, University of Tehran during the winter season 2005 – 2006. The experiment was laid out in factorial arrangement in randomized complete block design (RCBD) with three replications. The factors included in the experiment were: row spacing (20,30, 40 cm) and herbicides including broad-spectrum (2,4-D Butyl Ester 72 EC + Isoprotouron 75 WP @ 865 ml ha^{-1} and 865 g ha^{-1}, respectively), broad leaf herbicide (Buctril M 40 EC @ 1.25 l ha^{-1}) and a weedy check. The standard seed rate of 120 kg ha^{-1} was used. A standard dose of 120:70 N:P kg ha^{-1} was used in the form of urea and di-ammonium phosphate. Half the nitrogen and full dose of phosphorus was applied at the time of seedbed preparation, while remaining half of the nitrogen was applied at first and second irrigation. The herbicides were sprayed 40 days after sowing to control all germinated weeds. Data on individual observations were recorded using the following procedure: weed density for grasses and broad leaf weeds was determined 15 days after herbicides application. A quadrate of one m^2 made of iron wire was placed randomly in three places in each sub-plot and weeds were counted and then mean was calculated m^{-2}. Fertile spikelets $spike^{-1}$ was counted at the time of harvest from ten randomly selected spikes from each treatment and the mean spikelets $spike^{-1}$ were calculated. The grains $spike^{-1}$were counted by threshing the above spikes, counting the grains and subsequently computing the mean grains $spike^{-1}$. The grain yield (t ha^{-1}) was recorded by obtaining per plot yield in kg and subsequently converting the data into t ha^{-1}. The data on harvest Index (H.I) were obtained by using the following formula:

$$\text{H.I.} = \frac{\text{Economic yield (t ha}^{-1}\text{)} \times 100}{\text{(Biological yield (t ha}^{-1}\text{)}}$$

Economics of crop production: Cost of all operations/inputs included in the production of wheat crop was calculated ha^{-1} (i.e. manual labour, machine labour, animal labour, land rent, seed, fertilizers, herbicides, water rates etc.). Gross income for main product (grain) and by product (straw) was calculated. The net income (Profit ha^{-1}) was calculated by subtracting production cost from the gross income.

Analysis of variance and mean separation tests were applied according to the method described by Gomez and Gomez (1984) using the MSTAT-C computer software package.

3. Results and Discussion

3.1 Grasses weeds density (m^{-2})

The effect of herbicides, row spacing and interaction of herbicide with row spacing on grasses weeds density m^{-2} was highly significant. The lowest grasses weeds (22 m^{-2}) were recorded in row spacing 20 cm, followed by 30 cm (29 m^{-2}) and 40 cm row spacing (34 m^{-2}) (Table 1). Among herbicides minimum number of grasses weeds m^{-2} were recorded in treatments treated with broad-spectrum herbicide (12 m^{-2}) followed by broad leaved (35 m^{-2}) and weedy check (38 m^{-2}) [Table 1]. In the interaction of row spacing with herbicides, minimum grasses weeds (8 m^{-2}) were recorded in row spacing 20 cm with broad spectrum herbicide treated plots, while maximum (43 m^{-2}) were found in row spacing 40 cm with control treatment (Table 1). The lowest density m^{-2} of grasses weeds recorded in 20 cm row spacing might be due to more competition of wheat crop for development resources as compared to wider row spacing. These results were in agreement with the work of Marwat (2002a), Marwat *et.al*., (2002a), and Sarir (1998), who reported that minimum grasses weeds m^{-2} were recorded in narrow row spacing. While minimum number of grasses weeds in broad-spectrum

herbicide treated plots were due to the presence of isoproturon in broad-spectrum herbicide, which controlled grasses weeds. Minimum grassy weeds m^{-2} in the interaction of broad spectrum herbicide and 20 cm row spacing might be due to its best combination as less space was available for grassy weeds development and application of broad spectrum herbicide controlled grasses weeds. These findings are in agreement with Marwat et.al., (2002) and Marwat et.al., (2002a), who reported that interaction of broad spectrum herbicide and narrow row spacing suppressed weeds population more effectively.

3.2 Broad leaved weed density (m^{-2})

The effect of row spacing, herbicides and interaction of row spacing and herbicides on broad leaf weeds density m^{-2} were highly significant. Among row spacings, 20 cm row spacing had minimum broad leaf weeds (26 m^{-2}) followed by 30 cm (31.33 m^{-2}) and 40 cm (35.33 m^{-2}). While minimum broad leaf weeds (12 m^{-2}) were recorded in broad-spectrum herbicide treated plots followed by broad leaf herbicide (19.33 m^{-2}) and weedy check (61.33 m^{-2}), where no herbicide was used (Table 2). For row spacing x herbicides interaction, minimum (10 m^{-2}) and maximum (72 m^{-2}) broad leaf weeds m^{-2} were observed in row spacing 20 cm with broad-spectrum herbicide and row spacing 40 cm with control treatment, respectively (Table 2). Maximum number of broad-leaved weeds in treatments of wider row spacing might be due to more space available for weeds development, while narrow row spacing suppressed weeds growth. These results are in agreement with the work of Marwat et al. (2002), Marwat et. al., (2002a) and Khan et al. (2002), who concluded that with the closer row spacing (18 and 15 cm), the weed growth rate was lower, and light interception, crop growth rate and grain yield were higher than with the wider row spacing. Among herbicides, minimum broad leaf weeds were recorded in broad-spectrum herbicide treated plots, which might be due to efficient control of broad leaf weeds. These findings were in agreement with the results of Marwat (2002a) and Marwat et.al., (2002a) who found that broad spectrum herbicide (Isoproturon + 2,4-D) controlled weeds population more effectively as compared to grasses weeds killer or broad leaf herbicide used alone. Minimum broad leaf weeds recorded in the interaction of 20 cm row spacing and broad-spectrum herbicides might also be due to less space available for weeds growth in narrow row spacing, and also application of broad-spectrum herbicide controlled broad leaf weeds. These results were also in agreement with the work of Marwat (2002a) and Marwat *et.al*., (2002a) who enunciated that with the closer row spacing (15 and 20 cm), the weed growth rate was slower, and light interception, crop growth rate and grain yield were higher. The broad-spectrum herbicide (Isoproturon + 2,4-D) controlled weeds population more effectively as compared to grasses weeds killer or broad leaf herbicide used alone.

3.3 Spikelets $spike^{-1}$

The effect of herbicides on spikelets $spike^{-1}$was significant, while that of row spacing and interaction of herbicides with row spacing was non-significant. The maximum spikelets $spike^{-1}$were observed in broad spectrum herbicide (2,4-D+ isoproturon) treated plots followed by broad leaved herbicide and weedy check (Table 3). The highest spikelets $spike^{-1}$ in broad spectrum herbicide treated plots might be due to control of both grasses and broad leaved weeds density and consequently wheat crop solely used plant nutrients and other resources, which might have increased spikelets $spike^{-1}$. These findings are in agreement with the work of Marwat (2002) and Khan et al. (2001), who reported that maximum spikelets $spike^{-1}$ were the result of application of broad spectrum herbicide (2,4-D+ isoproturon) and (Puma + Logran).

3.4 Grains $spike^{-1}$

The effect of herbicides, row spacing and interaction of row spacing with herbicides were highly significant. The maximum grains $spike^{-1}$ (55.07) were recorded in broad spectrum followed by broad leaved (53.50) herbicide, while minimum grains $spike^{-1}$ (52.07) were recorded in the weedy check (Table 4). Among row spacings, the highest grains $spike^{-1}$ were found in 30 cm (54.73), followed by 20 cm (54.06), while minimum was recorded in 40 cm (51.83) row spacing. In row spacing x herbicides interaction, the highest grains $spike^{-1}$ were recorded in 30 cm row space treated with broad-spectrum herbicide (56.70), while minimum grains were counted in 40 cm row spacing with weedy check plots (51.20) (Table 4). Maximum grains $spike^{-1}$ in broad-spectrum herbicide treated plots might be due to the control of both grasses and broad leaved weeds and thus wheat crop might have used nutrients sufficiently, which could have ultimately increased number of grains $spike^{-1}$. These findings are in agreement with the work of Marwat (2002), who found that maximum grains $spike^{-1}$ were recorded in plots treated with broad spectrum herbicide. Maximum grains $spike^{-1}$ recorded in 30 cm row spacing might be due to suitable row space for higher grains $spike^{-1}$. These findings are in accordance with the work of Marwat (2002), who reported that maximum grains $spike^{-1}$ were found in row spacing 25 cm at Peshawar and row spacing 30 cm at Dera Ismail Khan.

3.5 Grain yield (t ha^{-1})

The effect of row spacing, herbicides and row spacing x herbicide on grain yield (t ha^{-1}) was highly significant. The highest grain yield was observed in 20 cm (4.80 t ha^{-1}) row spacing, followed by 30 cm (4.43 t ha^{-1}) and 40 cm (4.23 t ha^{-1}) respectively (Table 5). Among herbicide treatments, maximum grain yield (4.83 t ha^{-1}) was recorded in broad-spectrum herbicide, which was significantly different from broad leaf (4.45 t ha^{-1}) and control treatment plots

(Table 5). The effect of row spacing x herbicides interaction on grain yield was also significant. The highest grain yield (5.35 t ha^{-1}) was recorded in 20 cm row spacing treated with broad spectrum herbicide, while minimum grain yield (4.00 t ha^{-1}) was recorded in 40 cm row spacing with control treatment (Table 5). The maximum grain yield observed in 20 cm row spacing, broad spectrum herbicide and interaction of 20 cm row spacing x broad spectrum herbicides was due to the fact that productive tillers m^{-2} were more in row spacing 20 cm and broad spectrum herbicide as compared to other two row spacing (30 cm and 40 cm) and broad leaved and weedy check treatments. These results are in agreement with the work of Marwat (2002a), Marwat *et al* (2002) and Malik *et al* (1996) who reported that grain yield and straw yield were highest at 18 and 15 cm row spacing and decreased at wider row spacing. In case of broad spectrum herbicide, both narrow and broad leaf weeds were controlled by application of narrow + broad leaf herbicides and ultimately increased grain yield. These findings are in agreement with the work of Marwat (2002a), Marwat *et al* (2002) and Azad *et al* (1997) who reported that post-emergence application of isoproturon + 2,4-D was found to be the best treatment combination in reducing dry matter of weeds and producing the greatest straw and grain yield compared to control treatment.

3.6 Harvest Index (%)

The effect of row spacing, herbicides and row spacing x herbicides interaction on harvest index was significant. Among row spacing the highest harvest index (32.66) was recorded in 20 cm row spacing, followed by 30cm row spacing (31.09) and lowest harvest index was observed in row spacing 40 cm (Table 6). Maximum harvest index was found in broad spectrum (33.49), followed by broad leaf (31.29), while minimum was recorded in control treatment (Table 6). From the interaction of row spacing x herbicides, the highest harvest index was observed in row space 20 cm x broad spectrum herbicide (35.91), followed by row spacing 30 cm x broad spectrum herbicide (32.78), while lowest harvest index (29.10) was computed for row spacing 40 cm with control (Table 6). The highest harvest index recorded in row spacing 20 cm, herbicide broad spectrum and interaction of row spacing 20 cm x broad spectrum herbicide might be due to maximum productive tillers m^{-2} found in row spacing 20 cm and broad spectrum herbicide, which controlled both narrow and broad leaf weeds and the up take of maximum soil nutrients by wheat crop increased grain yield and thus harvest index was increased. These findings are in agreement with the work of Marwat (2002), who found that the higher harvest index was recorded in narrow row spacing and application of broad-spectrum herbicide.

3.7 Net income (Rs. ha^{-1})

The effect of herbicides, row spacing and herbicides x row spacing was significant. Table 7 revealed that maximum net income (Rs.29748 ha^{-1}) was found in broad-spectrum herbicide, followed by broad leaf (Rs. 26450 ha^{-1}), while minimum was in control treatment (Rs.24828 ha^{-1}). Among row spacing, maximum net income (Rs.29655 ha^{-1}) was recorded in 20 cm row spacing, followed by 30 cm (Rs.26538 ha^{-1}) and 40 cm (Rs. 24838.33 ha^{-1}), respectively (Table 7). Comparing the interaction, highest net income (Rs. 34140 ha^{-1}) was found in row spacing 20 cm treated with broad spectrum herbicide, followed by 30 cm row spacing (Rs. 28615 ha^{-1}) plots sprayed with broad spectrum herbicide, while minimum net income was found in 40 cm row spacing and with weedy check treatment (Table 7). The maximum net income recorded in broad spectrum herbicide might be due to the fact that broad spectrum herbicide containing both narrow and broad leaved herbicides, controlled both kinds of weeds effectively and grain and straw yield of wheat was increased which ultimately increased the net income. These findings are in agreement with the work of Marwat et al (2002a) and Kotru et al., (1999) who reported that post-emergence application of isoproturon + 2,4-D gave the highest benefit cost ratio of 2.57 and 1.38 and net profit of Rs.35350 and Rs.712.5 ha^{-1}, respectively. The highest net income recorded in row spacing 20 cm was due to maximum productive tillers m^{-2}, which increased both grain and straw yield of wheat and effectively increased net income. These findings are in agreement with the work of Marwat et al (2002a) and Pattanaik et al., (1996) who reported that closer unidirectional sowing + integrated weed management resulted in the highest net returns (Rs. 35433 and Rs. 5753 ha^{-1}, respectively) and the highest net return for each Rupee invested (Rs. 2.57 and 1.72, respectively). The narrow rowed sowing was superior in field and more economical.

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Table 1. Effect of row spacing, herbicides and their interaction on grasses weeds density m^{-2} in wheat

Herbicides	Row spacing (cm)			Herbicide means
	20	30	40	
Broad Spectrum	8 a*	12 b	16 c	12 a
Broad Leaf	27 d	35 f	43 g	35 b
Weedy check	31 c	40 g	43 g	38 b
Row spacing mean	22 a	29 b	34 c	
LSD value for row spaces, herbicide and row space x herbicide = 3.57				

* Means not sharing a common letter in the respective category differ significantly at 1 % level of probability.

Table 2. Effect of row spacing, herbicides and their interaction on broad leaved weeds density m^{-2} in wheat

Herbicides	Row spacing (cm)			Herbicide means
	20	30	40	
Broad Spectrum	10 a*	12 a	14 ab	12 a
Broad Leaf	18 bc	20 c	20 c	19.33 b
Weedy check	50 d	62 e	72 f	61.33 c
Row spacing mean	26 a	31.33 b	35.33 b	
LSD value for row spaces, herbicide and row space x herbicide = 4.26				

*Means not sharing a common letter in the respective category differ significantly at 1 % level of probability.

Table 3. Effect of row spacing, herbicides and their interaction on spikelets $spike^{-1}$ in wheat

Herbicides	Row space (cm)			Herbicide means
	20	30	40	
Broad Spectrum	19.60*	19.20	19.40	19.40 a
Broad Leaf	18.60	18.70	18.50	18.60 b
Weedy check	18.20	18.10	17.90	18.07 b
Row spacing mean	18.80 NS*	18.66	18.60	
LSD value for row spaces, herbicides and row space x herbicide = 0.59				

* NS: Non-significant. * Means not sharing a common letter in the respective category differ significantly at 1 % level of probability.

Table 4. Effect of row spacing, herbicides and their interaction on number of grains $spike^{-1}$ in wheat

Herbicides	Row spacing (cm)			Herbicide means
	20	30	40	
Broad Spectrum	56.10 a	56.70 a	52.40 d	55.07 a
Broad Leaf	54.10 bc	54.50 b	51.90 de	53.50 bc
Weedy check	52.00 d	53.00 cd	51.20 e	52.07 c
Row spacing mean	54.06 b	54.73 a	51.83 c	
LSD value for row spaces, herbicide and row space x herbicide = 1.47				

* Means not sharing a common letter in the respective category differ significantly at 1 % level of probability.

Table 5. Effect of row spacing, herbicides and their interaction on grain yield (t ha^{-1}) in wheat

Herbicides	Row space (cm)			Herbicide means
	20	30	40	
Broad Spectrum	5.35 a	4.70 b	4.45 c	4.83 a
Broad Leaf	4.70 b	4.40 c	4.25 cd	4.45 b
Weedy check	4.35 c	4.20 de	4.00 e	4.18 c
Row spacing mean	4.80 a	4.43 bc	4.23 c	
LSD value for row spaces, herbicide and herbicide x row space = 0.209				

* Means not sharing a common letter in the respective category differ significantly at 1% level of probability.

Table 6. Effect of row spacing, herbicides and their interaction on harvest index (%) in wheat

Herbicides	Row space (cm)			Herbicide means
	20	30	40	
Broad Spectrum	35.91 a	32.78 b	31.79 bc	33.49 a
Broad Leaf	31.97 b	31.10 c	30.80 cd	31.29 bc
Weedy check	30.10 d	29.41 de	29.10 e	29.83 c
Row spacing mean	32.66 a	31.09 ab	30.56 b	
LSD value for row spaces, herbicide and row space x herbicide = 1.66				

* Means not sharing a common letter in the respective category differ significantly at 1% level of probability.

Table 7. Effect of row spacing, herbicides and their interaction on net income (Rs. ha^{-1}) in wheat

Herbicides	Row space (cm)			Herbicide means
	20	30	40	
Broad Spectrum	34140	28615	26490	29748
Broad Leaf	28580	26030	24755	26450
Weedy check	26245	24970	23270	24828
Row spacing mean	29655	26538	24838	

* Means not sharing a common letter in the respective category differ significantly at 1 % level of probability.

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Land Use and Cover Mapping with Airborne Hyperspectral Imager in Setiu, Malaysia

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Abstract

In recent years, land use and land cover plays a pivotal role in global environmental change. Under these circumstances, the need of a new dimension for detecting land use and cover is getting more imperative for conservation and effective management of land use and cover types. Importantly, the use of information technology to support decision making in detecting land use and cover is essential and recent. One of the technologies used is Airborne Remote Sensing. The objective of this study is to identify, quantify, classify and map land use and land cover mapping in Setiu, Terengganu using UPM-APSB's AISA airborne hyperspectral remote sensing. Detection of land use and cover was performed using airborne hyperspectral imaging data taken on 20 April 2006 with the support of existing land use and cover maps. The size of the study area is 100 ha. The image was displayed in ENVI 4.0 Software using bands 202217 (RGB) combination. The data were then enhanced and classified for different land use and cover classes. From the data analysis, the image can be classified into eight classes. The classes are 2-3 years old oil palm plantation, 4-5 years old oil palm plantation, young (3-4 years old) rubber plantation, matured (15-17 years old) rubber plantation, vegetation crops, open area, road and river. The land use and land cover classes area distribution of the plots under study in Setiu, Terengganu were 4.18 ha, 8.58 ha, 6.26 ha, 70.43 ha, 2.98 ha, 2.31 ha, 2.78 ha, and 2.48 ha. Overall, the classification accuracy of interpretation of the airborne imagery for land use and cover in Setiu, Terengganu is 89.51 and kappa coefficient is 0.86. This study shows that, airborne hyperspectral remote sensing technique is capable in identifying, quantifying, classifying and mapping land use and cover in Setiu, Terengganu, hence a good decision support tool in land use and cover planning and management.

Keywords: Landuse, Mapping, Airborne, Hyperspectral, Precision agriculture, Rubber, Oil palm

1. Introduction

There are few landscapes remaining on the Earth's surface that have not been significantly altered or are not being altered by humans in some manner. Mankind's presence on the Earth and his modification of the landscape has had a profound effect upon the natural environment. These anthropogenic influences on shifting patterns of land use are a primary component of many current environmental concerns as land use and land cover change is gaining recognition as a key driver of environmental change (Riebsame, *et. al*., 1994). Changes in land use and land cover are pervasive, increasingly rapid, and can have adverse impacts and implications at local, regional and global scales.

During the past millennium, humans have taken an increasingly large role in the modification of the global environment. With increasing numbers and developing technologies, man has emerged as the major, most powerful, and universal instrument of environmental change in the biosphere today. Both globally, land cover today is altered primarily by direct human use (Meinel and Hennersdorf, 2002). Any conception of global change must include the pervasive influence of human action on land surface conditions and processes.

Viewing the Earth from an aerial view has become essential to comprehend the cumulative influence of human activities on its natural resource base. In a time of rapid, and often unrecorded, land use change, observations from the air provide near real-time objective information of human utilization of the landscape. Airborne hyperspectral remote sensing is providing new tools for advanced ecosystem land use and cover sustainable management. By utilizing

airborne hyperspectral sensing technologies land use and land cover change of designated areas can be monitored and mapped in near real time for specific research, analysis, quick and precise decision making. Other than that, it is very important for the government to know the status of the land in near real time. Developing methodology for interpreting the land use and land cover with combined with near real-time temporal airborne imageries and cadastre GIS datasets is deemed necessary. Manual (traditional) mapping methods acquire a relatively time-consuming and high expenditure. Pressing needs for land use inventory in many of the developed countries and some of developing countries have already prompted the use of airborne hyperspectral sensing and GIS to provide up-to-date information (Kamaruzaman and Norsuzila, 2008; and Kamaruzaman, 2008a, 2000b, 2000c)

The general objective is to asses the capability and usefulness of UPM-APSB's AISA airborne hyperspectral remote sensing in land use and cover mapping. The specific objective is to identify, quantify, classify and map land use and land cover mapping in Setiu, Terengganu.

2. Method and materials

2.1 Description of study area

The study was conducted in Setiu, Terengganu within latitude 5^0 28' 21.91" to 5^0 28' 4.07" North and longitude 102^0 49' 59.82 to 102^0 50' 54.58 East. Figure 1 shows the location of study area in Setiu, Terengganu. Setiu is one of the distinct in Terengganu. Total cover areas in Setiu are 130,436.3 ha. The total plantation areas in Setiu are 36,945.5 ha, including rice paddy, vegetables, fruits, cash crops, coconut, tobacco and others. The mean relative humidity of the study site is about 70% to 90%. The mean minimum and maximum temperature are 22^0C to 32^0C respectively. The annual rainfalls in Setiu fluctuated from the lowest 2990 mm to the highest of 4003 mm per year. 90% of the residents in Setiu are Malay, and the minority races consist of Chinese and Indian.

<<Figure 1. A map of Peninsular Malaysia show in the location of study area in Setiu, Terengganu >>

2.2 System description

UPM-APSB's AISA has been proven an accurate and reliable tool for collecting data. UPM-APSB's AISA is a solid-state, push-broom instrument. The small size of the complete system makes it quite portable for use in various aircraft. The instrument can be mounted on a plate that is compatible with a standard aerial camera mount, available in airplanes around the world. Other than that hyperspectral instruments is the flexibility in selecting the sensor's spatial and spectral resolution characteristics. Simple ASCII text configuration files that can be written and loaded at anytime control the sensor settings, bandwidth selections, and integration time. Multiple configuration files are loaded on the controlling computer and used interchangeably throughout the flight depending on the image targets or mission goals.

UPM-APSB's AISA is capable of collecting up to 288 spectral channels within this range, the data rate associated with the short integration times (sampling rates) required of the sensor in most operational/flight modes, limits the number of channels. The full spectral mode, however, is useful for acquiring 288 band spectral signatures of specific targets that can be used to generate pure end members as well as for band selection purposes. Current operational collection configurations range from 10 to 70 spectral bands depending on the aircraft speed, altitude and mission goals. The placement of the spectral bands is completely configurable and the user selected bandwidths can range anywhere from ~2nm to ~10nm.

2.3 Data acquisition and image processing

The raw data pre-processed image (Figure 2) has been analysed from the UPM-APSB's AISA airborne hyperspectral remote sensing on-board the aircraft. The image was used for land use and land cover classification. The data which is in digital format was obtained from UPM- Aeroscan Precision (M) Sdn Bhd.

<<Figure 2. A raw data image from the UPM-APSB's AISA airborne hyperspectral sensor>>

The image processing and analysis was done by a personal computer using The Environment for Visualizing Images (ENVI) 4.0 system. Data collection, analysis and ground verification are the three main components involved in this study. Manual classification involved visual observation based on differences in the spectral responses and image contrast. There are 20 bands used in the data analysis. A selection of band combination of the image was attempted in order to acquire the appreciate False Colour Composite Image (FCC) of the study.

For this study, 30 sampling point were selected randomly during the ground verification. Photographs were taken and parameters related to land use and land cover types to this study were recorded. All collected data obtained from ground verification were used to determine the accuracy of mapping. Accuracy assessment needs to ensure that the classification has been done was precise as to the real condition on the ground. Mapping accuracy was made through an error matrix. The pixel that has been categorized from the image was compared to the same site in the field. If the comparison is match, it would be put under similar category. The original references data were obtained from ground visits and topographic map. The reference data was simply done by calculating the percentage of the truly classified

pixels over total number of pixels that were presented in the confusion matrix. Meanwhile, the mean classification accuracy was calculated as the function of total training pixel against the total correct pixel over the total pixel.

3. Results and discussions

In this study, Bands 1-29 of airborne hyperspectral imaging were tested for selection of suitable band combination to get a good colour combination image that can give more information about land use and land cover. Image represented by band 20, 22 and 17 showed the best quality among all the airborne hyperspectral imaging bands. From the colour enhancement technique, agricultural area, open area, and road are easily recognized because of their dark colour against the light background of rubber plantation and oil palm plantation. Combination of bands 20, 22 and 17 (RGB) showed excellent result in identifying and differentiating land use and land cover pattern. Figure 3 showed the colour enhanced image which is much easier to recognize because the increasing of information for visually interpreted from the image.

<<Figure 3: Airborne hyperspectral image after enhancement showing land use and cover of Setiu, Terengganu >>

In this study, the enhanced image was classified using a SAM supervised classification. A total of eight different classes of land use and cover types were able to be classified and mapped as follows: Class 1: Oil palm plantation (2-3 Years), Class 2: Oil palm plantation (4-5 Years), Class 3: Young Rubber plantation (3-4 Years), Class 4: Matured Rubber Plantation (15-17 Years), Class 5: Agricultural crops (bananas, rubber saplings (1 year) and pumpkin), Class 6: Open area, Class 7: Road and Class 8: River. The result of the eight classes of land use and cover and the reflectance of spectral signature for this classification is shown in Figures 4-11.

<<Figure 4. Image and spectral signature for 2 – 3 years old oil palm plantation

Figure 5. Image and spectral signature for 4 – 5 years old oil palm plantation

Figure 6. Image and spectral signature for young (3-4 years old) rubber plantation
Figure 7. Image and spectral signature for matured (15-17 years old) rubber plantation

Figure 8. Image and spectral signature for agricultural crops (bananas, 1 year old

rubber plantation(1years) and pumpkin)

Figure 9. Image and spectral signature for open area

Figure10. Image and spectral signature for road

Figure 11. Image and spectral signature for river>>

Based on the image and spectral signature in Figures 4-11, it can be seen that every class of land use and cover have their own reflectance of spectral signature. The difference in reflectance of spectral signature can be used to classify the land use and land cover. Agricultural crops classes have higher reflectance of spectral signature. This is perhaps due to the lower absorption of light compared to other land use and cover types. The nutritional deficiency in the vegetation usually decreases absorption and increase reflectance and transmittance in the visible wavelength. So, the increasing of nutritional in agricultural crops gives higher reflectance of the spectral signature. On the other hand, the study by Ourcival (1999) found that development factor such as leaf age and plant species are also can caused dissimilar reflectance spectra. Besides, the reflectance of spectral signature for young (3-4 years old) rubber plantation are higher compared to matured (15-17 years old) rubber plantation. The decrease of chlorophyll concentration is a factor that effects this situation. The low level of chlorophyll should be due to the normal ageing process. Iron, magnesium, nitrogen and sunlight are necessary for chlorophyll production, so lack of any one of these can lead to yellowing of leaves (Kupiec and Curran, 1993) and provided the decreasing reflectance in spectral signature. Open area, river and road classes have low reflectance of spectral signature. The decrease of open area spectral reflection is depending on organic content, moisture contents and various other minerals. For example, as soil becomes moist, its reflectance decreases. Thus, river and road is strong absorber of reflectance energy in the infrared portion of the electromagnetic spectrum. The reflectance of river and road is generally low. The field photographs taken for each landuse and cover during ground verification is illustrated in Figures 12-17.

<<Figure 12. Ground photos for a young (L) and matured (R) oil palm plantations>>

<<Figure 13. Ground photos for a young (L) and matured (R) rubber plantations>>

<<Figure 14. Ground photos for a mixed agriculture (L) and an open area (R)>>

<<Figure 15. Ground photos for a road (L) and a small muddy river (R)>>

Table 1 showed the total amount of land use and land cover classes of the study area in Setiu, Terengganu. The image point out that, they were eight land use and land cover classes. The classes were 2-3 years old oil palm plantation, 4-5 years old oil palm plantation, young (3-4 years old) rubber plantation , matured (15-17 years old)rubber plantation, agricultural crops, open area, road and river The total area and percentage of every land use and land cover are counted

according to the total pixels for each class by using the software. The major land use and land cover classes area distribution of Setiu, Terengganu were 2-3 years old oil palm plantation (4.18%), 4-5 years old oil palm plantation (8.58%), young (3-4 years old) rubber plantation (6.26%), matured (15-17 years old) rubber plantation (70.43%), agricultural crops (2.98%), open area (2.31%), road (2.78%), and river (2.48).

<<Table 1. Land use and cover classes of the study area in Setiu, Terengganu>>

The result was assessed for accuracy after completing the classification procedure. The results for accuracy assessment were shown in the Table 2 using confusion matrix. The overall accuracy was 89.51% which is higher than qualification accuracy of a digital classified image of 80%. Kappa coefficient was 0.86. Kappa analysis is discrete multivariate technique of use in accuracy assessment (Congalton and Mead, 1983). Table 3 shown the producer's and user's of classification.

<<Table 2. Confusion matrix of the image classification for the study area>>

<<Table 3. Producer's and user's accuracy of the image classification for study area>>

Table 3 showed that the highest producer's accuracy was road which achieved 97.78%. Others classes were in the average of 85%, such as 2-3 years old oil palm plantation (95.60%), 4-5 years old oil palm plantation (87.64), young(3-4 years old) rubber plantation (84.18%), matured (15-17 years old) rubber plantation, agricultural crops (95.55%) and river (94.95%). The lowest producer's accuracy was the open area (97.78%). Road class hit the highest user's accuracy of 97.78%. Road were easier to be identified due to the highest reflection than the surrounding rubber plantation, oil palm plantation and agricultural crops. The open area class had the lowest accuracy of 72.49% due to misclassification of agricultural crops during image classification. The user's accuracy of 2-3 years old oil palm plantation, 4-5 years old oil palm plantation, young (3-4 years old) rubber plantation , matured (15-17 years old) rubber plantation, agricultural crops, open area, road and river were 89.31%, 89.31%, 78.78%, 97.72%, 81.12%, 92.62%, 74.87%, and 90.30%.

4. Conclusion

It can be concluded that the UPM-APSB's AISA is capable and useful for detecting, identifying, quantifying classifying and mapping land use and land classes with an overall accuracy was 89.51% and Kappa coefficient was 0.86. The 100 ha size study area of Setiu comprise of land use and cover with 2-3 and 4-5 years old oil palm plantation, a 3-4 (young) and 15-17 (matured) years old rubber plantation, mixed agricultural crops, open areas, roads and rivers. It is quite evident from this study that the most of villagers planted rubber for their income with an areal extent of 70.43 ha for matured rubber and 6.26 ha for young rubber followed by oil palm (4.18 ha for 3-4 years old and 8.58 ha for 15-17 years old), 2.98 ha for mixed agricultural crops, 2.31 ha for open areas, 2.78 ha for roads and 2.48 ha for rivers.

Airborne hyperspectral sensing is a potential tool for monitoring and estimating agricultural land use and cover because it can provide quicker information than traditional mapping method. Development should attempt to operationalize airborne hyperspectral sensing intensively for future planning and control of the sustainable agricultural land use and land cover development in Setiu, Trengganu, Malaysia.

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Table 1. Land use and cover classes of the study area in Setiu, Terengganu

Class	Area (Hectares)	Percentage(%)
2-3 years old oil palm plantation	4.18	4.18
4-5 years old oil palm plantation	8.58	8.58
Young (3-4 years old) rubber plantation	6.26	6.26
Matured (15-17 years old) rubber plantation	70.43	70.43
Agricultural crops	2.98	2.98
Open area	2.31	2.31
Road	2.78	2.78
River	2.48	2.48
TOTAL	100.00	100.00

Table 2. Confusion matrix of the image classification for the study area

Overall accuracy = (16294/18203) 89.51% Kappa Coefficient = 0.86									
Ground Truth Pixels									
CLASS	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**TOTAL**
A	**1195**	82	0	53	0	0	0	8	1338
B	0	**1525**	0	380	0	0	0	2	1907
C	7	4	**1240**	292	0	11	8	12	1574
D	40	119	8	**7529**	0	0	0	9	7705
E	0	0	137	0	**967**	77	0	11	1192
F	0	0	15	1	13	**904**	18	25	976
G	0	1	47	33	31	245	**1147**	28	1532
H	8	9	26	138	1	10	0	**1787**	1979
TOTAL	1250	1740	1473	8426	1012	1247	1173	1882	**18203**

A = Young oil palm plantation
B = Matured oil palm plantation
C = Young rubber plantation
D = Matured rubber plantation
E = Agricultural crops
F = Open area
G = Road
H = River

Table 3. Producer's and user's accuracy of the image classification for study area

Classes	Producer's Accuracy		User's Accuracy	
	Pixel	Percentage (%)	Pixel	Percentage (%)
2-3 years old oil palm plantation	1195/1250	95.60	1195/1338	89.31
4-5 years old oil palm plantation	1525/1740	87.64	1525/1907	89.31
Young (3-4 years old) rubber plantation	1240/1473	84.18	1240/1574	78.78
Matured (15-17 years old) rubber plantation	7529/8426	89.35	7529/7705	97.72
Agricultural crops	967/1012	95.55	967/1192	81.12
Open area	904/1247	72.49	904/976	92.62
Road	1147/1173	97.78	1147/1532	74.87
River	1787/1882	94.95	1787/1979	90.30

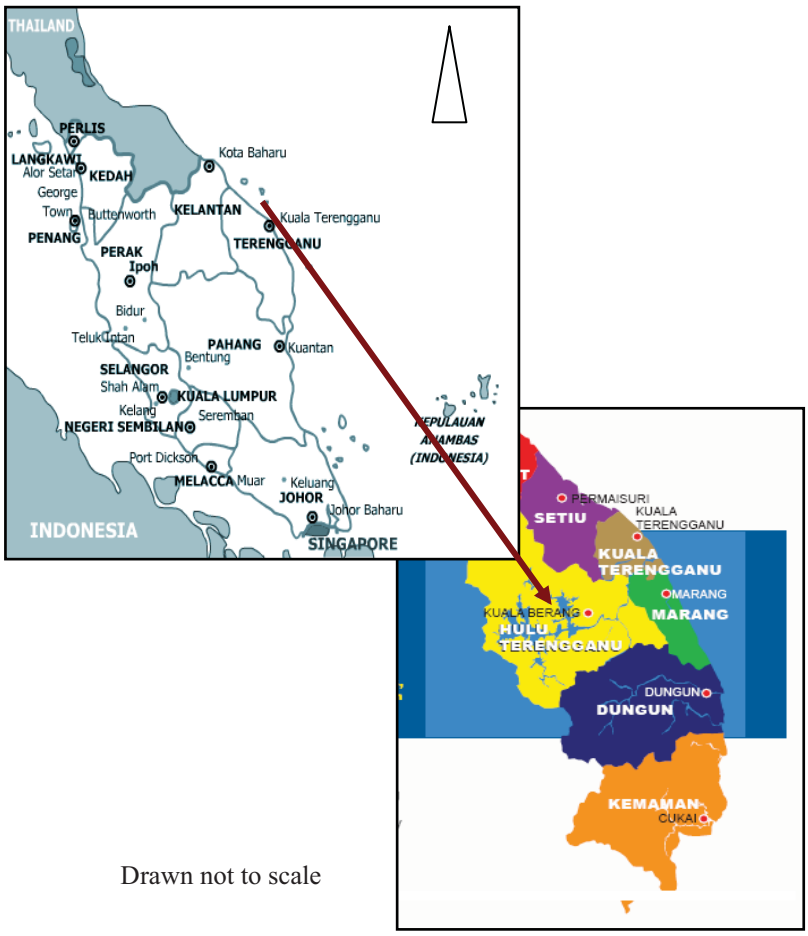

Figure 1. A map of Peninsular Malaysia show in the location of study area in Setiu, Terengganu

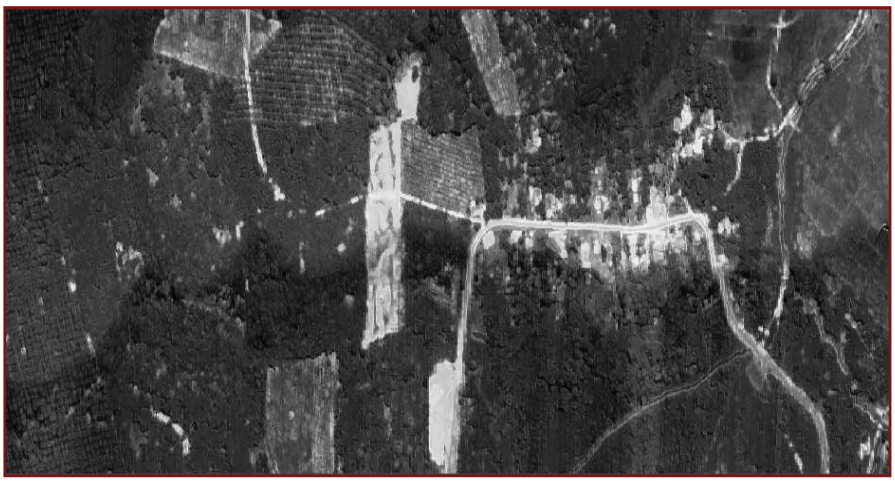

Figure 2. A raw data image from the UPM-APSB's AISA airborne hyperspectral sensor

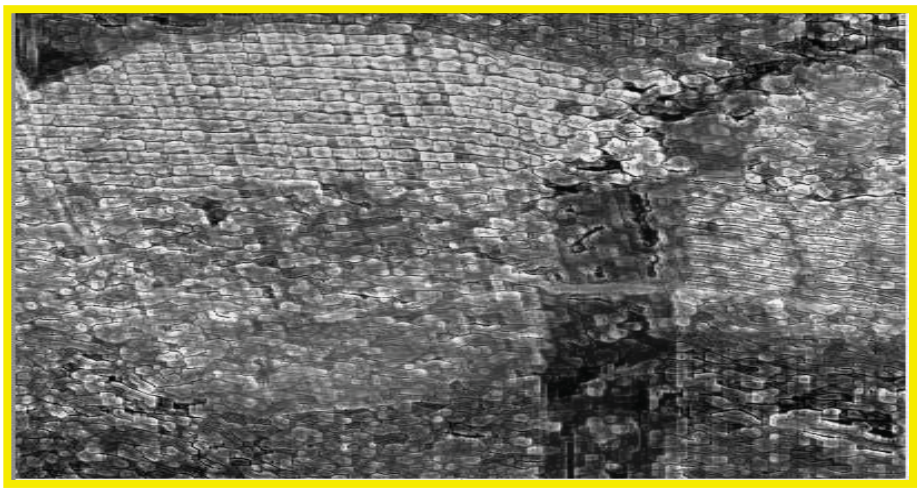

Figure 3. Airborne hyperspectral image after enhancement showing land use and cover of Setiu, Terengganu

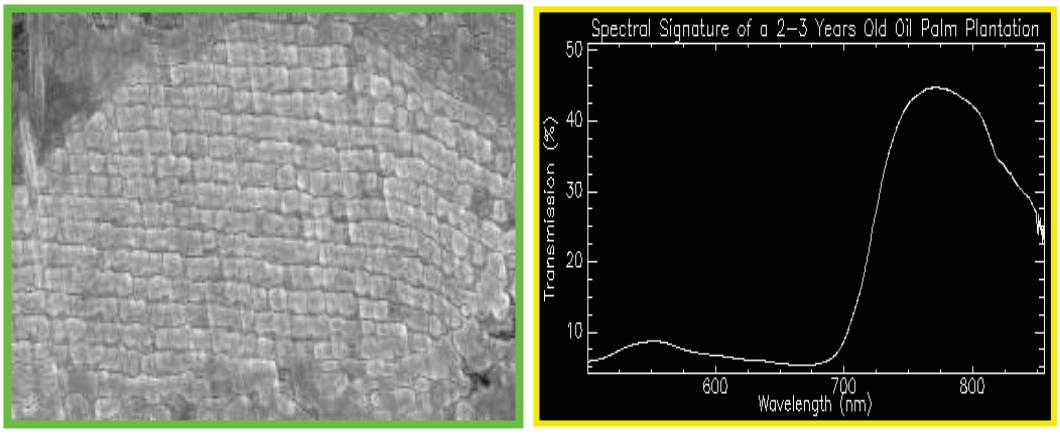

Figure 4. Image and spectral signature for 2 – 3 years old oil palm plantation

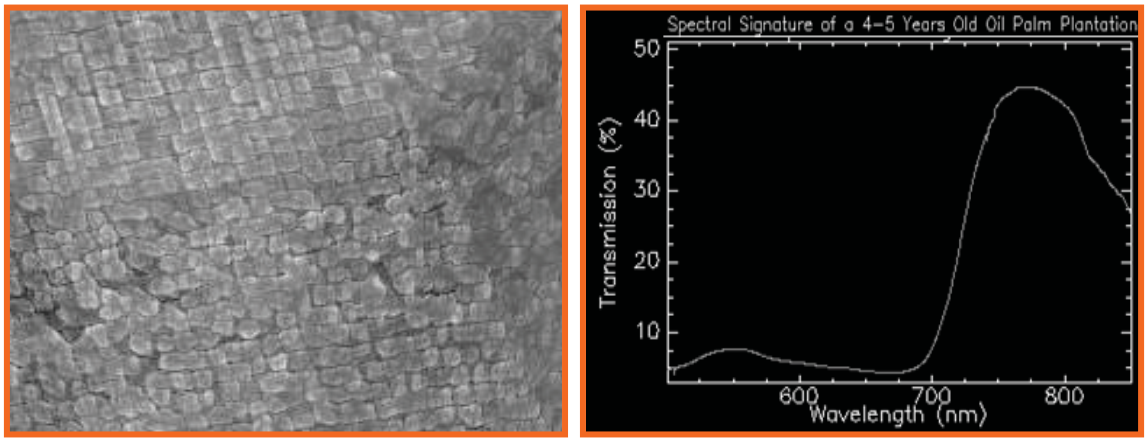

Figure 5. Image and spectral signature for 4 – 5 years old oil palm plantation

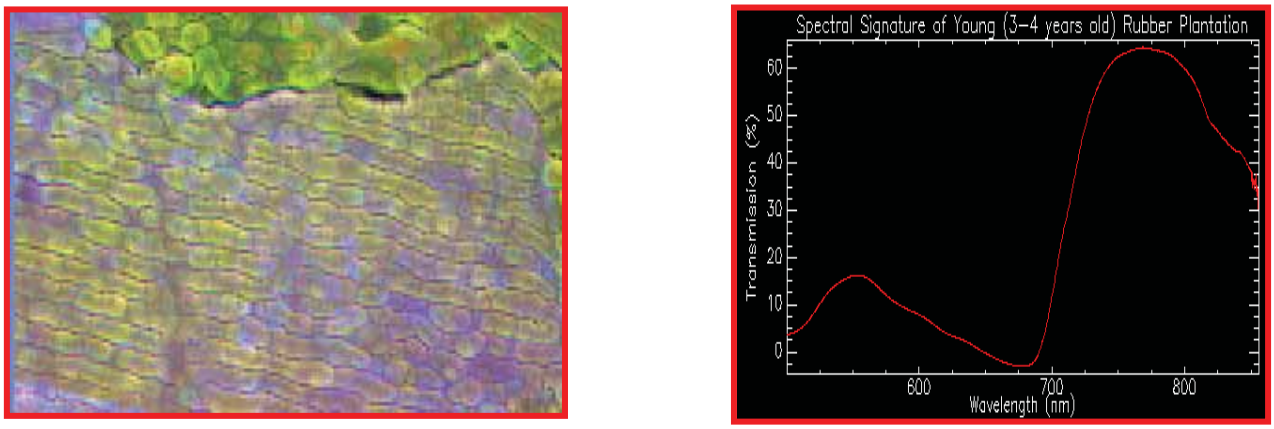

Figure 6. Image and spectral signature for young (3-4 years old) rubber plantation

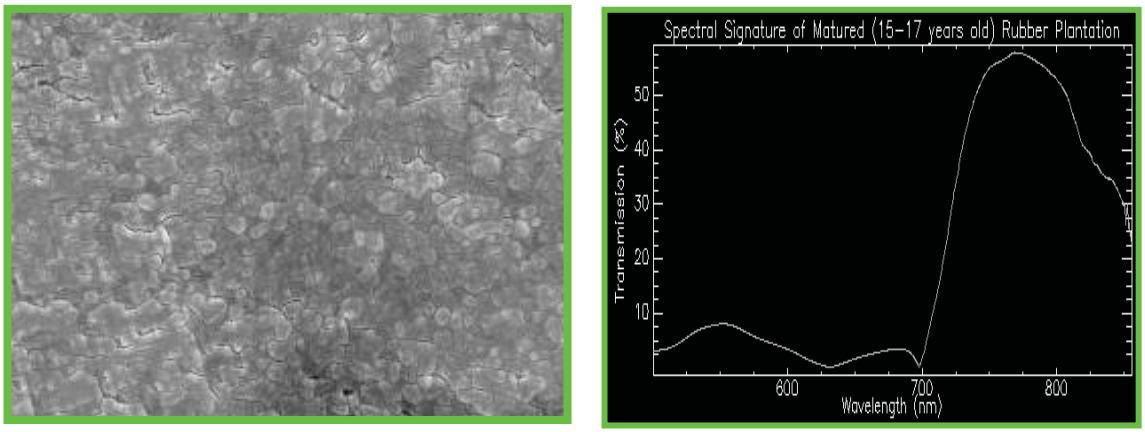

Figure 7. Image and spectral signature for matured (15-17 years old) rubber plantation

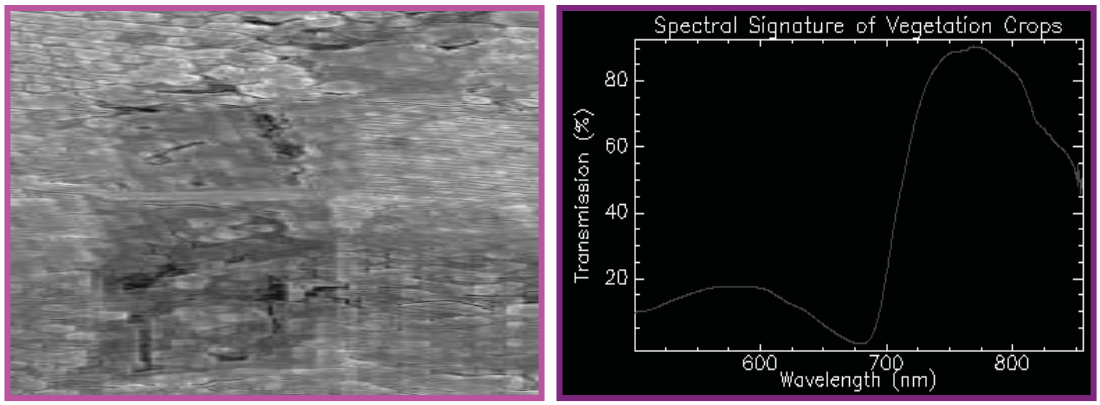

Figure 8. Image and spectral signature for agricultural crops (bananas, 1 year old rubber plantation and pumpkin)

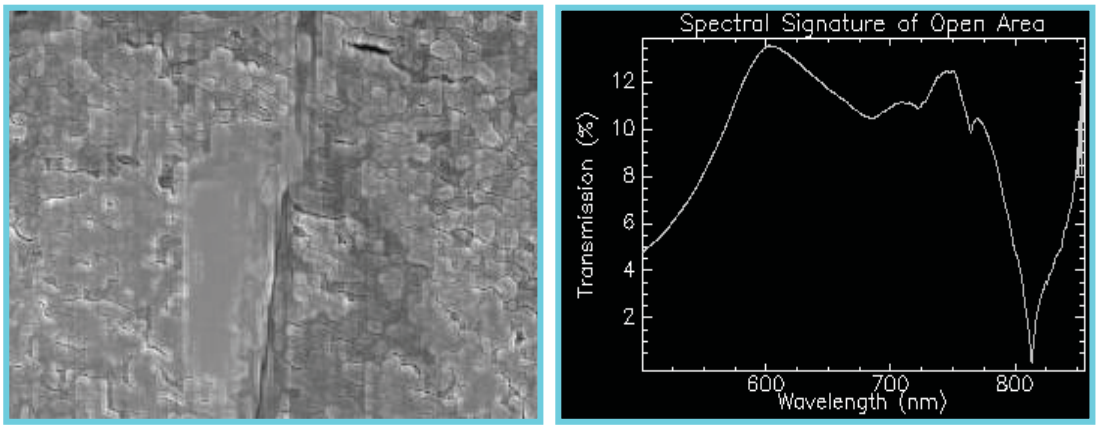

Figure 9. Image and spectral signature for open area

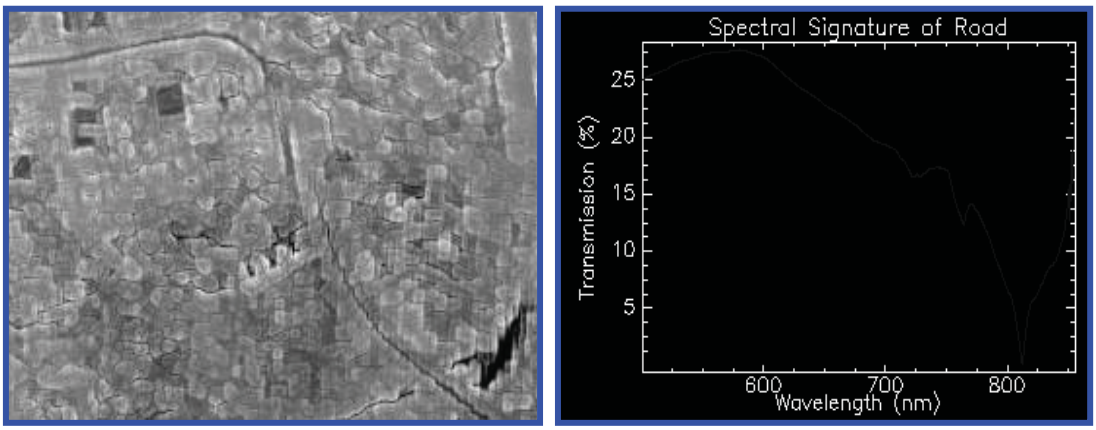

Figure 10. Image and spectral signature for road

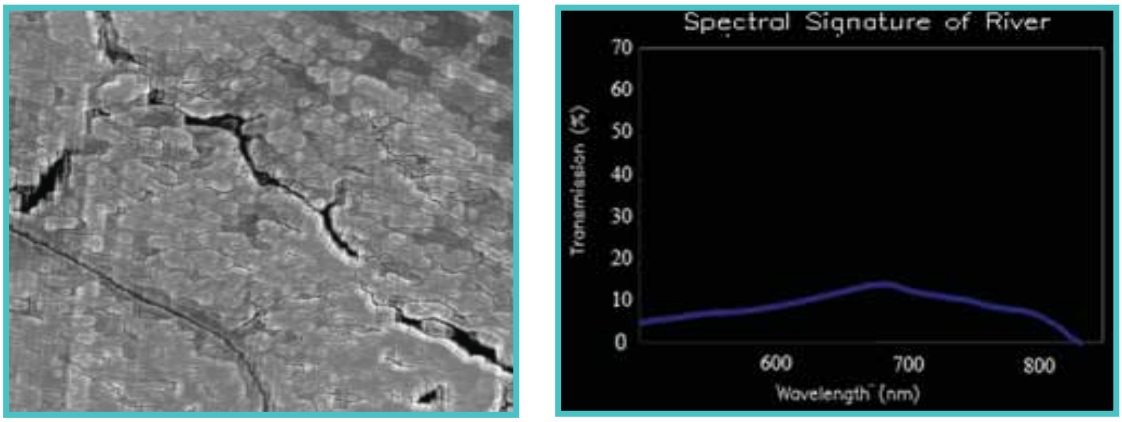

Figure 11. Image and spectral signature for river

Figure 12. Ground photos for a young (L) and matured (R) oil palm plantations

Figure 13. Ground photos for a young (L) and matured (R) rubber plantations

Figure 14. Ground photos for a mixed agriculture (L) and an open area (R)

Figure 15. Ground photos for a road (L) and a small muddy river (R)

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The Biocontrol Effect of Trichoderma and Bacillus Subtilis SY1

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Abstract

As years of unreasonable farming practices in agriculture soil damaged seriously. The soil-borne disease and the chemical residue are two serious problems of soil pollution which affect the yield and quality of agricultural products. Ecological remediation of soil is an effective way to resolve these problems. In this paper, the agents' trichoderm and the Bacillus subtilis SY1 were used to improve the ecosystem function and reduce the disease occurrence.

Keywords: Biocontrol effect, Trichoderma, Bacillus subtilis SY1

1. Introduction

The sensitivity of Ginseng to pathogenic fungus has increased after being domesticated out of wildness state. It is reported that there are more than thirty kinds of diseases harmful to Ginseng's growth statistically which can aggrieve its roots, flowers, fruits and could result in fall of production, quality of seeds and roots(Charron.D., Gagnon. D., 1991; W. G. Bailey. 1996,).

Except for the chinese herbal medicine, the problem of plant fungal pathogens are common in agriculture nowadays, especially in facility agriculture. As a new direction for biological control method has recently been developed and beneficial agents, such as Bacillus subtilis (Shinji Mizumoto et al, 2007; Wang J et al, 2007; Marc Ongena et al, 2005) are widely used.

In this paper, trichoderm and Bacillus subtilis SY 1 were used in the experiments to determine their biocontrol effect to pathogen fungi in soil. trichoderm a could antagonize several typical pathogen fungi of American ginseng and decrease the disease occurrence. The eggplant planting experiments indicated that Bacillus subtilis SY1 not only could antagonize several pathogen fungi of eggplant but could promote the seedling growth and increase its stress tolerance.

2. Materials and method

2.1 Microbial agents

The Bacillus subtilis SY1 used through this work was preserved in the laboratory.

Nine pathogenic fungi of American Ginseng and one anti-biotic fungus Trichoderma were separated in the Ginseng planting base.

The four vegetable plant pathogens are provided by Vegetable Research Institute, Tianjin Academy of Agriculture Sciences.

2.2 Experimental method

2.2.1 American ginseng experiment

2.2.1.1 The confrontation experiment

In the confrontation experiment, on the PDA media a piece of 5 mm diameter antagonistic fungi (3 days old cultures) was put on one side and the pathogen fungi (7 days old cultures) of the same size was put on the other side, 4cm apart from each other. In the control experiment 5mm diameter pathogen fungi were put on the PDA media. All the experiments were repeated 5 times The plates were incubated at 28±1℃ for 7 days to detect the antibacterial effect.

$$I = \frac{R_2 - R_1}{R_2} \times 100\% \quad (1)$$

I—Antibacterial effect;

R1—Straight-line distance between the center of the pathogen fungi and the edge of antagonistic fungi;

R2—The radius of the control pathogen fungi.

2.2.1.2 American ginseng field experiment

The field experiment of inoculated and control were undertaken in the American ginseng planting base. The inoculated and the control were undertaken simultaneously and were both repeated three times. After disinfecting, the ginseng roots were regularly arranged on the land which surface soil was removed in advance. After covering the soil back onto ginsengs, 5 mL trichoderm suspension was added as liquid to the roots of the American ginseng. Three times of inoculation were did in the first three month after sowing. The desease occurrence were determined after 12 month.

2.2.2 Eggplant experiment

2.2.2.1 Antagonistic examination

In vitro antagonistic examination of the antifungal activity of Bacillus subtilis SY1 was tested against the four typical soil-borne pathogen diseases on the PDA media. Spore suspensions with different fungi (5 days old cultures, the concentration was more than 106 cfu / mL) have been prepared in 0.85% sterilized saline. The melted solid wateragar medium was put on the plates, the sterile stainless steel columns were put on the frozen solid agar. When the melted semisolid PDA media cooled to 40-50℃, 1 mL of spore suspensions of each fungus was put in and well-mixed with PDA media. The mixture was then put onto the wateragar medium plates using transfer pipet. When the agar was frozen solid, the stainless steel columns were taken away and 50 μL transformation was put into the hole except the controls (put into 50 μL sterilized saline). Every treatment was repeated three times. The plates have been incubated at 28±1℃ for 7 days to detect the diameter of fungal inhibition around.

2.2.2.2 Eggplant field experiment

2.2.2.3 Eggplant seedling growth promoting examination

Eggplant seeds were disinfected with 70% ethanol -water ratio solution and then with 0.5% sodium hypochlorite. After rinsing six times with sterile water, the seeds were soaked in the water to pregermination. After three days, eggplant seeds were selected and sown into the pots. The plants were irrigated with water every other day. After 20 days, the growth situation and antioxygen enzyme of eggplant seedling were determined.

2.3 Analysis method

Chlorophyll content——By means of ethanol extraction

Superoxide dismutase (SOD)——By means of nitroblue tetrazolium photoreduction

Catalase (CAT) ——By means of ultraviolet absorption

Peroxidase (POD) ——By means of guaiacol method (Wu et al, 2006)

3. Results and discussion

3.1 American ginseng experiment

3.1.1 The confrontation experiment

3.1.2 American ginseng field experiment

The American ginsengs in each block were dug out to be detected one years later, and the root diseases were classified

according to its condition. Table 2 shows the ginseng root diseases rate.

root diseases classification:

Grade 0: ginseng root anosis

Grade 1: small disease spots on the ginseng root surface

Grade 2: disease spots on 1/5 of the surface

Grade 3: disease spots on 1/5 of the surface

Grade 4: 1/2 of the root rot

Grade 5: most of the root rot

$$Disease\ rate\ \%=100-\ Grade\ 0\ ginseng\ number\ /total\ ginseng\ number\times 100 \quad (2)$$

3.2 Eggplant experiment

3.2.1 The antagonistic examination

In vitro antagonistic examination of the antifungal activity of Bacillus subtilis SY1 was tested against the four typical soil-borne pathogen diseases on the PDA media. Spore suspensions with different fungi (5 days old cultures, the concentration was more than 106 cfu / mL) have been prepared in 0.85% sterilized saline. The melted solid wateragar medium was put on the plates, the sterile stainless steel columns were put on the frozen solid agar. When the melted semisolid PDA media cooled to 40-50℃, 1 mL of spore suspensions of each fungus was put in and well-mixed with PDA media. The mixture was then put onto the wateragar medium plates using transfer pipet. When the agar was frozen solid, the stainless steel columns were taken away and 50 μL transformation was put into the hole except the controls (put into 50 μL sterilized saline). Every treatment was repeated three times. The plates were incubated at 28±1℃ for 7 days to detect the diameter of fungal inhibition around.

From Table 3 and Fig 1 we can see that Bacillus subtilis SY1 has antifungal activity to all these four pathogens to a certain extent, especially D Alternaria solani the diameter of fungal inhibition ring attained 43.6 mm. Alternaria solani is the most common and harmful soil-borne pathogen fungi in agriculture which causes decline of plant yield and quality every year. Bioremediation of inoculating beneficial bacteria Bacillus subtilis SY1 is an effective method to decrease the pathogen fungi amount. The lower quantity and activity of soil-borne pathogen fungi could decrease the disease occurrence so as to guarantee a bumper harvest.

3.2.2 Eggplant field experiment

The eggplants growth condition were determined after 3 months, and the diseases condition were classified. Table 2 shows the eggplants diseases rate.

disease investigation:

Grade 0: plant anosis

Grade 1: few small disease spots on the leaves and the stems anosis

Grade 2: disease spots on most of the leaves and the stems anosis

Grade 3: most of the leaves turn yellow but the stems anosis

Grade 4: most of the leaves turn yellow and the stems rot

Grade 5: withered plant

$$disease\ index\ \%=\sum(disease\ plant\ numbers\ of\ each\ grade\times grade\ number)/(total\ plant\ numbers\times 9)\times 100 \quad (3)$$

3.2.3 The growth situation and antioxygen enzyme of eggplant seedling

After 20 days' growth, eggplantseedlings selected in random order were dug out and cleaned with sterile water and plant height, dry weight, chlorophyll content, antioxidant enzyme such as SOD, CAT and POD were determined. The results are shown in Table 2.

From Table 2 we can clearly see that the growth of seedling and the antioxidant enzyme activities in the plant all improved after inoculating the Bacillus subtilis SY1. The Plant height and the dry weight of the seedling increased 56.61 % and 33.55 % respectively. The improvement of chlorophyll content and antioxidant enzyme activities stands for the increase of plant metabolism and stress tolerance. The chlorophyll content is closely related with photosynthesis which provide energy to the plant. SOD is a common enzyme that exists throughout the animal and plant kingdoms, it could remove the superoxide radical. The activity of CAT has a great impact on plant resistance to cold and disease. POD is a highly active enzyme that also common exists in the plant which is closely related to the respiration, photosynthesis and degradation reaction of growth hormone.

4. Conclusions

In the American ginseng planting experiment the biocontrol aggent trichoderm has great antifungal effect to several pathogens that common in American Ginseng planting soil. After inoculating the trichoderm, the disease occurrence deased significantly.

In the eggplant planting experiment the Bacillus subtilis SY1 has great antifungal effect on pathogens and the growth and stress resistance of the seedlings in the inoculated soil increased. After inoculating, the plant height, dry weight and chlorophyll content increased by 56.61%, 33.55% and 40.1% respectively. The antioxidant enzymes SOD, CAT and POD improved significantly by 103.2 %, 127.3% and 81.5 %.

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Table 1. Antibacterial effect of trichodermto American ginseng fungous diseases

Pathogenic fungi	R_1/cm	R_2/cm	$I= (R_2-R_1)/R_2\times100\%$
F_1 *Alternaria panax*	2.5	9	72.2
F_2 *Fusarium sp.*	1.5	5	70.0
F_3 *Phytophthora cactorum*	1.7	9	81.1
F_4 *Cylindrocarpon sp.*	2	9	77.8
F_5 *Acremoniella cucurbitae Schulz1et Saccl*	2.0	7	71.4
F_6 *Erysiphe panacis Bai et Wang*	1	4	75.0
F_7 *Monilia cinerea Bon*	2	8	75.0
F_8 *Bot rytis cinerea Persl*	2.6	9	73.3
F_9 *Phoma panacicola Nakata et Takimoto*	2.2	8	72.5

Table 2. The disease rate of each block

		Grade 0	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5	Zero-grade rate %	Disease rate%
Block: 1	control	9	16	3	0	0	0	32.14	67.86
	inoculated	17	12	0	0	0	0	58.62	41.38
Block: 2	control	9	15	3	0	0	0	33.33	66.67
	inoculated	13	14	0	0	0	0	48.15	51.85
Block: 3	control	7	19	4	0	0	0	23.33	76.67
	inoculated	16	12	0	0	0	0	57.14	42.86

Table 3. Inhibitory activities of Bacillus subtilis SY1 to four typical pathogens

	Pathogenic bacteria	Diameter of fungal inhibition ring (mm)
A	*Pythium aphanidermatum*	22.4
B	*Fusarium oxysporum f.sp.lycopersici*	20.2
C	*Botrytis cinerea Pers.*	16.5
D	*Alternaria solani(Ell.et Mart.)*	33.6

Table 4. The disease rate of each block

		Grade 0	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5	Disease rate%
Block: 1	control	39	5	2	0	1	3	6.2
	inoculated	45	3	1	0	0	1	2.2
Block: 2	control	40	6	1	0	2	1	4.7
	inoculated	47	2	0	0	1	0	1.3

Table 5. The growth situation and antioxidant enzyme activities of eggplant seedling after 20 days

Treatment	Plant height (cm)	Dry weight (mg)	Chlorophyll Content ($mg \cdot L^{-1}$)	SOD ($U \cdot g^{-1}$)(FW)	CAT ($U \cdot g^{-1}$)(FW)	POD ($U \cdot g^{-1}$)(FW)
Control	7.912±0.169	4.717±0.173	0.536±0.134	86.210±0. 210	21.075±0.208	35.883±0.100
Inoculated	12.390±0.103	6.300±0.009	0.751±0.125	175.150±0. 195	47.906±0.106	65.122±0.251
Increasing rate (%)	56.61	33.55	40.1	103.2	127.3	81.5

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